

A roadmap for investigation and validation of Dry Fogging as a decontamination technology

Prepared by:
Samantha Kimball
Calian Technologies Ltd
101
340 Legget Dr
Ottawa, ON K2K 1Y6

Paul Bodurtha and Eva F. Gudgin Dickson
CBRN Protection Group
Dept. of Chemistry & Chemical Engineering
Royal Military College of Canada
13 General Crerar Crescent
Kingston, ON K7K 7B4

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CSA: Norman Yanofsky, Portfolio Manager, Chemistry, (613) 944-8161

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A Roadmap for Investigation and Validation of Dry Fogging as a Decontamination Technology

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Samantha Kimball¹, Paul Bodurtha^{1,2}, Eva F. Gudgin Dickson²

1. Calian Technologies, Ottawa, ON.
2. CBRN Protection Group, Dept. of Chemistry & Chemical Engineering, Royal Military College of Canada, Kingston ON; Calian

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Abstract

Decontamination following a chemical, biological, radiological, or nuclear (CBRN) event involves a well-integrated approach to contain and, just as importantly, clean up after the threat. Decontamination of a biological event occurs in three stages: the immediate, critical incident response; the consequence management phase; and remediation to reinstate the area to a level deemed safe for human re-occupation. Decontamination efforts typically include cleaning and disinfection of material objects, equipment, people and the surrounding environment. Methods for large area decontamination used currently, for example gaseous formaldehyde, have several drawbacks including carcinogenicity and environmental toxicity of the agent employed, labour intensiveness of the process, corrosiveness of the agent to metal surfaces and its incompatibility with electronic equipment. Dry fogging system (DFS) technology has been identified as a potential safe, robust, portable and efficacious decontamination method for biological agents, both for targeted threats and in industries where decontamination of biological contaminants is a routine concern.

Although such systems have been commercialized and fielded, the technology readiness level (TRL) for dry fogging systems for application in emergency response is estimated at between levels 4 and 7, based on the significant knowledge gaps on capabilities in many actual applications of interest, due to lack of appropriate validation activities. Therefore further research both at the laboratory level and in the field is required to validate DFS for operationalization. This report will outline the knowledge gaps and identify strategies to ensure maturity of DFS for first responders in routine practice and in a CBRN event. The objectives listed below are addressed in this report to support implementation of DFS technology. Several stakeholders, including the Royal Military College of Canada (RMCC), participated in identifying the needs for knowledge transfer and operationalization of DFS in different organizations. These objectives are as follows:

- Review current practices of decontamination in several industries: in emergency response vehicles (Ottawa Paramedic Service), in the food industry in food processing plants and on farms (Canadian Food Inspection Agency), and for military use (Canadian Forces);
- Review the available evidence to support the use of commercially available DFS for large area decontamination;
- Identify the knowledge gaps that need to be addressed to operationalize DFS technology;
- Identify and engage potential project partners who could benefit from the use of DFS technology, identify where needs cross-over occurs, and build a collaborative scheme to address the research knowledge gaps identified; and,
- Outline appropriate studies necessary to mature DFS technology.

The operationalization of DFS technology offers many potential advantages over current decontamination practices, both in routine practices and in a potential CBRN event scenario.

Résumé

La décontamination en cas d'événement chimique, biologique, radiologique ou nucléaire (CBRN) requiert une démarche intégrée de circonscription du danger et, chose non moins importante, de nettoyage après la menace. La décontamination à la suite d'un événement biologique se déroule en trois étapes : la réaction immédiate à l'incident critique; la gestion des conséquences; et la prise de mesures correctrices visant à rétablir un degré de sécurité jugé suffisant pour la réoccupation de la zone par des êtres humains. Les efforts de décontamination comprennent habituellement le nettoyage et la désinfection des objets, de l'équipement, des personnes et de l'environnement. Les méthodes actuelles de décontamination d'une grande zone, par exemple le recours au formaldéhyde sous forme gazeuse, présentent plusieurs inconvénients, notamment la cancérogénicité et l'écotoxicité de l'agent employé, les besoins importants en main-d'œuvre, la corrosivité de l'agent pour les surfaces métalliques et l'incompatibilité de l'agent avec l'équipement électronique. Les systèmes de décontamination à brouillard sec ont été pressentis comme technologie pouvant s'avérer sûre, résistante, portable et efficace en cas de contamination biologique, que ce soit pour une utilisation contre des menaces ciblées ou dans des industries où les contaminants biologiques constituent une préoccupation courante.

Bien qu'il existe déjà des systèmes de décontamination à brouillard sec sur le marché, on estime que le stade de développement de cette technologie appliquée aux interventions d'urgence se situe entre les niveaux 4 et 7, compte tenu des lacunes considérables, dues au manque d'activités de validation adaptées, en matière de connaissance des capacités dans de nombreuses applications d'intérêt. Il faudra donc mener des études plus poussées, aussi bien en laboratoire que sur le terrain, pour valider les systèmes à brouillard sec en vue de leur opérationnalisation. Le présent rapport fait état des lacunes en matière de connaissances et présente des stratégies pour assurer la maturité des systèmes à brouillard sec en vue de leur utilisation par les premiers intervenants dans leur pratique courante ou en cas d'événement CBRN. Les objectifs énumérés ci-dessous sont abordés dans le rapport à l'appui de la mise en œuvre de la technologie des systèmes à brouillard sec. Plusieurs intervenants, dont le Collège militaire royal du Canada (CMRC), ont participé à la détermination des besoins en matière de transfert des connaissances et d'opérationnalisation des systèmes à brouillard sec au sein de différentes organisations. Les objectifs sont les suivants :

- La révision des pratiques de décontamination courantes dans plusieurs industries : véhicules d'urgence (services ambulanciers d'Ottawa), industrie alimentaire, c.-à-d. usines de transformation des aliments et fermes (Agence canadienne d'inspection des aliments), et secteur militaire (Forces canadiennes);
- Le survol des preuves concrètes de l'utilité des systèmes à brouillard sec offerts sur le marché pour une utilisation à grande échelle;
- La détermination des lacunes à combler sur le plan des connaissances nécessaires à l'opérationnalisation de la technologie des systèmes de décontamination à brouillard sec;
- Le repérage d'éventuels partenaires de projet susceptibles de s'intéresser à la technologie des systèmes de décontamination à brouillard sec, l'établissement de liens avec eux, la détermination des domaines dans lesquels les besoins se recoupent, et la mise sur pied d'une structure de collaboration pour aborder les lacunes cernées en matière de connaissances;
- L'aperçu des études nécessaires au perfectionnement de la technologie des systèmes de décontamination à brouillard sec.

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L'opérationnalisation de la technologie des systèmes de décontamination à brouillard sec pourrait présenter de nombreux avantages par rapport aux pratiques de décontamination actuelles, que ce soit dans un contexte d'activités courantes ou dans des scénarios d'événement CBRN.

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1. Introduction

The Centre for Security Science (CSS) biological cluster has identified decontamination of biological hazards to be a significant research priority. A number of knowledge gaps were identified by participants from the scientific community and the first responder community at two separate Decontamination Workshops held by CSS in the fall of 2012 [1][2]. Participants at each workshop identified knowledge gaps in the realm of decontamination science, operations and policy. Importantly, recurring gaps in the science that supports decontamination were identified at both workshops. The gaps include the limitations surrounding our ability to respond to a biological attack for example in the arenas of health care, emergency services and food industries. Identified areas of improvements include the use and operationalization of new technology. Dry fogging systems were identified as an emerging technology to be matured for biological decontamination.

The intent of this document is to provide a roadmap for investigation of dry fogging, a potential technology with broad application to decontamination of biological hazards, which could be applicable to various types of events such as disease outbreaks and against the deliberate use of a biological agent.

Current cleaning and disinfection practices are manual. Personnel in the various arenas in which biological contamination is a routine problem typically wipe, mop and apply antimicrobial solutions by hand, with or without personal protective equipment (PPE), as necessary. The limitations of these practices are that:

- they are time consuming and subject to human error;
- fumes can lead to health concerns while PPE to mitigate is burdensome;
- hand wiping often leaves hard to reach areas contaminated;
- they require frequent changes of wipes due to spread of contamination by the cleaning implement;
- they can be corrosive to equipment, and contamination of equipment is a concern; and
- these methods often are not sufficient to decontaminate more resistant microorganisms, such as spores.

Dry fogging technologies offer a potential system of decontamination that addresses these deficiencies.

Dry fogging systems (DFS) disperse fine droplets (aerosol) of a disinfectant into a sealed room or other area. This method offers several potential advantages above manual cleaning and disinfection practices. These portable systems release controlled and consistent amounts of disinfectant into the air, removing the element of human error. Disinfectant is delivered remotely and the area can be rapidly exhausted of disinfectant so that no human exposure occurs. Disinfection reach is extended because the mist penetrates inaccessible areas (hard to reach places) and covers virtually every surface. Turnaround time is improved because the systems are much more rapid than manual methods. DFS can employ solutions containing peracetic acid which is effective against a broad spectrum of microbes, non-corrosive, non-toxic and environmentally safe and hence has particular applicability when the organism to be decontaminated is unknown or needs to be applied in the field.

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Although there are several DFS systems available on the market, the supporting evidence of system efficacy is lacking. This roadmap will review the available technology and the disinfectants available for use with each system, the potential applicability of DFS and summarize the necessary steps to operationalize DFS. Further, this roadmap will identify research knowledge gaps in several different industries where dry fogging systems would be beneficial and outline a collaborative scheme to address the required studies to validate the use of DFS in its various capacities. The Royal Military College of Canada (lead) will build partnerships with Public Health Agency of Canada, Canadian Food Inspection Agency, Environment Canada, Ottawa Paramedic Service and the Canadian Forces to address the knowledge gaps found and to perform required research activities to mature DFS technology.

2. Principles of Disinfection

Decontamination and disinfection protocols for biological agents vary significantly depending on the application. For example, requirements of the food industry are very different from those of paramedic services. No single disinfectant is adequate for all situations and disinfection protocols can differ based on the need, such as the containment of an infectious disease outbreak. The identity of the microorganism of concern and the chosen disinfectant both contribute to the effectiveness of decontamination, along with the conditions under which the procedure will be performed.

This section will first describe the biological agents of concern and disinfection principles and practices to provide the reader with a background of the challenges faced in the decontamination process. The requirements for decontamination of different working environments will also be discussed.

2.1 Disease Transmission

The word “germ,” “bacterium” or “virus” may trigger a reflex to wash one’s hands. Generally, a biological hazard, or agent, is a microorganism (bacteria, viruses, rickettsia, fungi and microscopic algae) or the product of a microorganism (e.g. a toxin). Most microorganisms do not cause disease in humans. Microorganisms that are capable of causing a disease are “pathogens.” To be a threat to human or animal health there are several factors that are required for a pathogen to cause infection, referred to as the chain of infection. These elements include a pathogen, a reservoir, microbial spread, a route of entry, a susceptible host and a route of exit (Figure 1; discussed in further detail in Annex B).

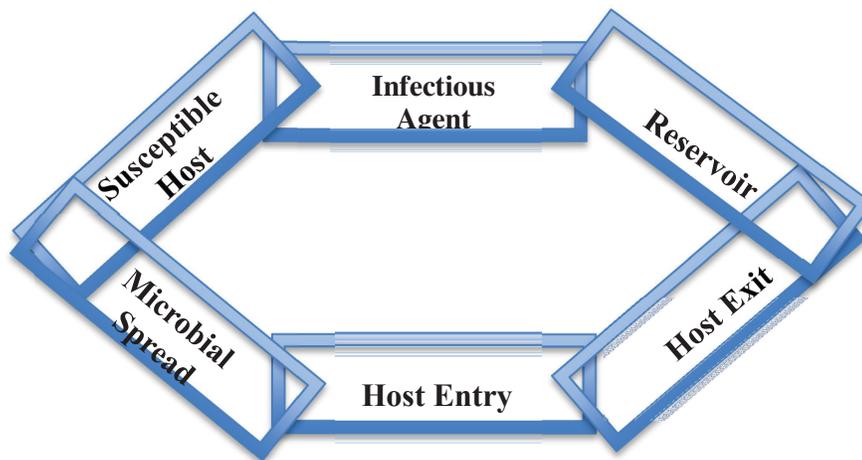


Figure 1: Chain of Infection; the links represent factors that are necessary for an infectious agent to survive, multiply and be passed onto another host

Microbial spread may be airborne, through direct contact, fecal-oral transmission or by way of a vector. Airborne microbes live in the nasal passages, making the nose run and triggering the sneeze reflex to initiate its own release. The sneeze produces a fine spray of microbe-bearing droplets that remain airborne for a sufficiently long time to enable it to spread to another individual. Airborne particles containing the microbe can also be found on environmental surfaces, a table or door handle, and be picked up on the hands of another person, and via hand to mouth contact find its way back to its targeted tissue. Direct contact, from person to person, is the route of transmission for microorganisms that are too fragile to survive in the environment for long. Gastrointestinal infectious agents travel between hosts via fecal

contamination of food and drinking water. These microbes reproduce in the gut and cause diarrhea that acts to flush the microbe back into the environment where it can infect the next host. Vectors can also be a mechanism of microbial spread. For example, an animal can carry a pathogen that can infect a human that comes into contact with it.

In some instances, a microorganism can be spread by a number of routes and can infect a variety of hosts (animals and humans). Zoonoses are diseases that occur in animals, with symptoms or not, that can be transmitted to humans. Certain diseases, such as anthrax, may infect both animals and humans. Anthrax spores can be spread by air, soil and animal; infection with anthrax spores can occur by inhalation, ingestion or through a skin lesion. Decontamination efforts must take all routes of microbial spread into account and eliminate reservoirs to prevent the spread of pathogens.

2.2 Sources of Infectious Agents of Relevance to Decontamination Issues

2.2.1 Hospitals

Pathogens can be found in high concentrations in hospitals. Common hospital acquired infection (HAI)-associated pathogens include *Pseudomonas*, *Acinetobacter baumannii*, and strains of common bacteria that are resistant to antibiotics such as Methacillin Resistant *Staphylococcus aureus* (MRSA) or Vancomycin Resistant *Enterococcus* (VRE). *Clostridium difficile* is a bacterium that produces highly resistant spores allowing it to survive in harsh environments for extended periods of time and a persistent source of hospital acquired infections (HAI). Disinfection practices within the hospital setting are of paramount importance for the prevention of HAI.

As recently as 2005 it was estimated that 1 in 10 patients get HAI and of those 1/3 was preventable [3]. Several factors perpetuate the spread of these microorganisms in the hospital environment, including: i) the abundance and variety of microorganisms in this environment; ii) compromised health status of individuals in hospital; iii) the chain of transmission in the hospital; and iv) the increased likelihood of encountering a resistant strain of pathogen.

Acinetobacter baumannii is a very common cause of HAI. These infections are often very difficult and costly to treat and have a mortality rate as high as 75% in some institutions [4]. These opportunistic organisms can cause urinary tract infections, pneumonia, meningitis, endocarditis, septicemia and cellulitis. *A. baumannii* is growing increasingly resistant to multiple antibiotics due to a wide array of resistance mechanisms – rapid evolution with environmental selective pressure [5]. These resistant bacteria often infect critically ill patients [6]. *A. baumannii* outbreaks in hospitals have been demonstrated [7]. *Acinetobacter* species are ubiquitous in the environment (soil, water, milk, frozen soups) and some adults have skin colonization. *Acinetobacter* presents an extra challenge in the hospital setting as it may colonize both environmental and skin surfaces and can survive for many months.

One of the most common infections present in hospitals and long-term care facilities is *Clostridium difficile*. *C. difficile* is the most common cause of gastrointestinal infection that follows antibiotic therapy or cancer chemotherapy, particularly in the elderly, ranging from mild diarrhea to severe or even fatal colitis. Rates of *C. difficile* HAI are increasing in adults and children [8]. Following antimicrobial therapy or chemotherapy, many organisms in the gastrointestinal tract are killed allowing *C. difficile* to multiply and produce abundant quantities of toxins that are responsible for intestinal disease with symptoms of bloody diarrhea, fever and severe abdominal pain. The bacteria are found in the feces of infected individuals and transmitted by fecal-oral contact. The next host becomes infected through touching

objects, surfaces, or the infected person that are contaminated with fecal traces, and then touching their mouth or nose.

Clostridium difficile is a species of gram-positive spore-forming bacteria. Endospores, or ‘spores’, are a dormant form of the bacteria that consist of the bacterium’s DNA, some cytoplasm and a very tough outer coating. Endospore formation allows the bacteria to lie dormant for extended periods and to survive harsh environments. When the environment is favourable again the endospore can reactivate itself to a vegetative state. Spores are resistant to UV radiation, desiccation, high temperature, extreme freezing, and some chemical disinfectants, which makes them very hard to eliminate in the environment. Their resistance to common disinfection practices ensures their survival in the hospital environment and contributes to their spread among susceptible individuals.

Refer to Annex C for more information on the organisms causing HAI.

2.2.2 Food Industry

Bacterial contamination of food is an area of growing concern – every few weeks there seems to be another recall of contaminated food products such as hamburger, spinach and tomatoes. Food can become contaminated anywhere along the food chain from growing, harvesting, post-harvest handling, transport, distribution, storage, packaging or during final preparation for distribution. Some of the major known pathogens that are involved in food-borne illness outbreaks and food recalls include *Salmonella* species, *E. coli* 0157:H7, *Listeria monocytogenes*, *S. aureus*, and Norovirus. The contamination of fresh produce is most commonly caused by contamination with *Salmonella* or *E. coli* 0157:H7 [9].

Disease outbreaks in domestic livestock can be devastating. Microbes that cause epidemics typically spread rapidly and lead to the destruction of whole flocks or herds once infection takes hold. Some of these diseases could be spread intentionally with comparative ease.

An example of one such disease is avian influenza (AI), caused by Influenza A subtypes that belong to the Orthomyxoviridae family of viruses. AI is a contagious viral disease that affects many species of birds including chickens and turkeys. There are multiple strains of the AI virus and variable degrees of illness, but H5 and H7 strains can cause outbreaks that require control or eradication. Occasionally, such strains can also cross over into the human population.

AI is transmitted to a flock of domestic poultry or turkey typically through direct contact with infected migratory waterfowl, their feces or by chicken to chicken contact. AI is highly contagious and the virus is found concentrated in feces and in nasal and eye discharge of infected animals. Poultry can be infected through contact with contaminated water, feed, vehicles, equipment, egg flats/cases, clothing or footwear. Fortunately, AI is very susceptible to common disinfectants and heat. Canadian Food Inspection Agency (CFIA) policy is to establish a “control area” around the farm to limit spread and infected flocks are destroyed.

Other examples of disease in livestock and poultry include foot and mouth disease (FMD) and bovine spongiform encephalopathy (BSE).

These industries require strictly efficient decontamination and disinfection processes, processes that are important to prevent illness in humans and animals. Prevention of disease involves breaking the chain of transmission (Figure 1) thus preventing the spread of infectious agents. Routine disinfection practices can eliminate the reservoir of infection and break that chain of infection and prevent morbidity and mortality caused by the spread of pathogens. Cleaning and decontamination efforts are extensive on a large farm

and incorporate live animals, carcasses, animal housing, equipment, electrical equipment, water, feed, machinery, vehicles, clothing, and manure.

Refer to Annex C for more information on the organisms causing food-borne illnesses.

2.2.3 Bioterrorism

Bioterrorism is defined as the use of any disease-causing organism or toxin found in nature with the intent of harm in civilian society. The use of biological agents for terrorism has been fortunately rare, one recent example being the 2001 anthrax attacks via the US postal service. Bioterrorism is generally recognized as a low to moderate probability, high impact terrorism event.

Bioterrorism agents are typically found in nature but may be altered to increase spread, survival of the organism or increase its virulence (capacity to cause disease). *B. anthracis* (in its spore form) and *Yersinia pestis* (causing pneumonic plague by human-to-human transmission) are two of the most significant concerns for bioterrorism relating to both likelihood and the severity of resulting effects, although a number of other agents are considered Category A bioterrorism agents by the US Centers for Disease Control (CDC) [10]. These agents can be variously spread via air, through contact with people or surfaces, or by ingesting intentionally contaminated water or food. Biological agents are extremely difficult to detect and may therefore go unnoticed until symptoms of contracted disease appear and spread is already considerable.

Anthrax was employed in the bioterrorism attacks in the United States in 2001 [11]. *B. anthracis* spores were distributed through the postal system to political and media targets. The anthrax letter attacks were relatively small, in that they consisted of only 7 mailed spore-containing letters, but the consequences were far-reaching. This included shutting down the US Congress, Supreme Court and postal service. The number of cases of inhalation anthrax that resulted from this attack, nineteen, again relatively few, but the impact economically (health care, decontamination and lost productivity) and psychologically was much greater. The total estimated cost of these attacks was \$320 million USD and was largely absorbed by decontamination and remediation efforts [12].

In comparison with chemical or explosive terrorist attacks, the impact of biological agents is more likely to be covert and to go unrecognized until symptoms emerge en mass. Consequently, the most likely first responders to a biological attack are health care providers. The result may be an undeterred spread of the agent before the attack has even been recognized.

Other agents have the potential for use in bioterrorism, the CDC classifies bioterrorism agents based on how easily they can be spread and the severity of illness or death they cause.

Category A agents are the highest risk and include organisms and their toxins. These agents have the potential to be easily spread or transmitted from person to person and result in high death rates with the potential for major public health impact. Category A diseases/agents include:

- Anthrax (*Bacillus anthracis*)
- Botulism (*Clostridium botulinum* toxin)
- Plague (*Yersinia pestis*)
- Smallpox (*Variola major*)

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- Tularemia (*Francisella tularensis*)
- Viral hemorrhagic fevers (e.g. filoviruses such as Ebola and Marburg and arenaviruses such as Lassa and Machupo)

Although *B. anthracis* spores are much harder to decontaminate than the other Category A agents, reduction of transmission by appropriate decontamination would still be highly pertinent if they were used in a bioterrorism incident.

Category B agents are moderately easy to disseminate and can cause moderate rates of morbidity and mortality and include brucellosis, ricin, food safety threats (for example, *Salmonella* species, *E.coli* O157:H7, and *Staphylococcus aureus*). Category C agents are the third highest priority and include emerging pathogens that could be engineered for mass spread in the future because they are readily available and easily produced and spread. The coronavirus responsible for severe acute respiratory syndrome (SARS), H1N1 influenza virus and hantavirus are examples of category C agents.

Refer to Annex C for further details on botulism and ricin toxins, plague, tularemia and viral hemorrhagic fevers.

2.3 How Clean Is Clean Enough?

Before we can choose the most appropriate method for the cleaning and disinfection of an area we must first ask what the acceptable level of residual contamination is, i.e. how clean is clean enough?

The answer to the question ‘how clean is clean’ is largely dependent upon the identity of the biological agent and the efficacy of the decontamination method. Each pathogen has an “Infectious Dose,” the number of microorganisms necessary to cause infection. The infectious dose varies between individuals and is dependent upon many factors, including: virulence of the microbe (its tendency to cause disease), route of entry and the health status of the host. Infectious dose varies greatly among microorganisms (one to thousands or millions). For example, the infectious dose of *B. anthracis*, which causes anthrax, is approximately 200-8,000 spores in humans [13], whereas the enterotoxin that causes staphylococcal food poisoning can cause symptoms at a toxin dose of less than 1.0 µg when ingested [14].

The level of exposure, i.e. acute versus chronic exposure, and the method of detection should also be considered. Acute, single low-level exposure (e.g. in one dish of food), versus chronic or continuous exposure (e.g. to an airborne pathogen that is present in the ventilation (HVAC) system) greatly influences the likelihood of reaching the threshold of an infectious dose. Further, determination of what level below the infectious dose must also take into account methodology sensitivity and specificity. It does not do us any good to set an acceptable level of contamination below that which we can measure with any degree of accuracy. The nuances include background counts, efficacy of recovery methods and the limits of detection of the quantitation method employed.

In the face of a biological attack, there are difficulties of translating laboratory practices into the field. In the lab we must simulate CBRN events, but with very important limitations: we do not use live agents but simulants whose characteristics may be similar some aspects but inherently different in others¹ and we contain our testing environment (confined to the lab). We cannot mimic those threats that pose the most

¹ e.g. *B. atrophaeus* in place of *B. anthracis*; although they are both gram positive, spore forming bacteria with a similar shape and size and of the same genus *Bacillus*, *B. atrophaeus* is generally more resistant to disinfectants than *B. anthracis* [15].

danger (e.g. *B. anthracis* aerosol release). Therefore, we cannot assume that the methods established in the lab will be easily adapted into the field or that they will be as effective. We do know from experience with large-scale farm decontamination (CFIA), that there are problems with maintaining a “clean” responder due to the practicalities of doffing PPE. This problem is not as pronounced in laboratory experiments where the conditions are more controlled and personnel are not exhausted from being in the field for extended periods.

There is also the issue of time. In the face of a biological attack, the attack may not be recognized until a terrorist group has claimed responsibility or large numbers of people develop symptoms of the disease. The decontamination scope exponentially increases with time from release due to spread. Determination of the most effective method for decontamination must also be decided quickly.

Decontamination processes must take all of these concerns into consideration. Ideally a disinfection practice would establish a level of “clean” that would pose the least amount of risk of infection for all agents (i.e. the level that is below the infectious dose of the most pathogenic agent, i.e. spores), would be easily adaptable to the field (portable and easy to use), would be rapidly effective, and would also pose the least amount of hazardous risk to personnel and the environment.

2.4 Disinfection methods

2.4.1 Cleaning

Cleaning, the removal of foreign material (e.g. soil, blood and body fluids), is normally performed using water and detergents or enzymatic products. Thorough cleaning is required before the process of disinfection or sterilization because foreign materials, organic or inorganic, can interfere with the effectiveness of these processes. Sterilization and disinfection can be carried out by either physical or chemical means, and these are discussed in general terms below, while further detail on all types of disinfection processes can be found in Annex C.

2.4.2 Physical Disinfection Methods

Physical means of disinfection include the use of heat, filtration, and radiation. Although useful for equipment and small areas, physical disinfection methods are not sufficient for large area decontamination.

2.4.3 Chemical Disinfection Methods

Chemical agents can be used for low to high-level disinfection and sterilization, depending on the chemical and its intended use. There are many factors that affect the activity of a disinfectant, including:

- i) characteristics of the surface (such as porosity and finish) – influences the reach of the disinfectant, for example, the microbes can be in all the small grooves that may not be penetrated by the chemical agent;
- ii) temperature – extreme temperatures can affect the efficacy of the disinfectant;
- iii) pH – characteristic of the chemical disinfectant;

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- iv) relative humidity – can influence activity of disinfectants, particularly gaseous chemicals, and can have an effect on their activity on environmental surfaces by impacting the rate of evaporation of water;
- v) water hardness - the presence of select cations, shown to decrease the activity of some microbicides;
- vi) presence of organic matter (e.g. blood, bodily fluids, soil) – proteins and lipids can act as a protective layer for microbes; and
- vii) the population of microorganisms present – the diversity of microorganisms can directly or indirectly influence their susceptibility to microbicides, for example, spores are more resistant than vegetative forms of bacteria. Differences in structures of microbes or viruses influence their susceptibility. Further, expression of “resistance” mechanisms may occur. Figure 2 attempts to classify the hierarchy of resistance to chemical disinfectants of different types of microorganisms.

These factors limit the use of each chemical agent and also serve as the characteristics by which to select the most appropriate disinfectant based on one’s need.

Disinfectant types vary by their chemical composition that determines the mechanism of action of the agent against microorganisms. The main classes of disinfectants used for environmental disinfection include: alcohols, aldehydes, chlorine compounds, iodophors, oxidizing agents, phenols and quaternary ammonium compounds. The mechanisms by which chemical agents exert their killing effect include:

- i) reacting with elements of the bacterial cell wall to disrupt integrity;
- ii) denaturing cellular proteins to disrupt transport across the cell membrane;
- iii) reacting with thiol (-SH) groups of enzymes to inactivate them; and
- iv) damaging DNA and RNA, which inhibits replication of the organism.

Disinfectants are not interchangeable. There are many classes of disinfectants that differ in their chemical make-up and, thus, their mechanism of action as discussed in further detail in Annex D. Contact time is an important factor for disinfectant action. If the contact time is too short, the killing function is not reached. Most disinfectants require a minimum contact time of 10 minutes for killing activity. For more resistant organisms, spores for example, longer contact times are required.

Typically, chemical disinfectants can be delivered as neat liquids, as solutions or foams, as aerosols (fogs) and as vapours, depending on the nature and size of the target surface as well as the type of disinfectant.

Table 1 summarizes each type of disinfectant along with its advantages and disadvantages.

Least susceptible

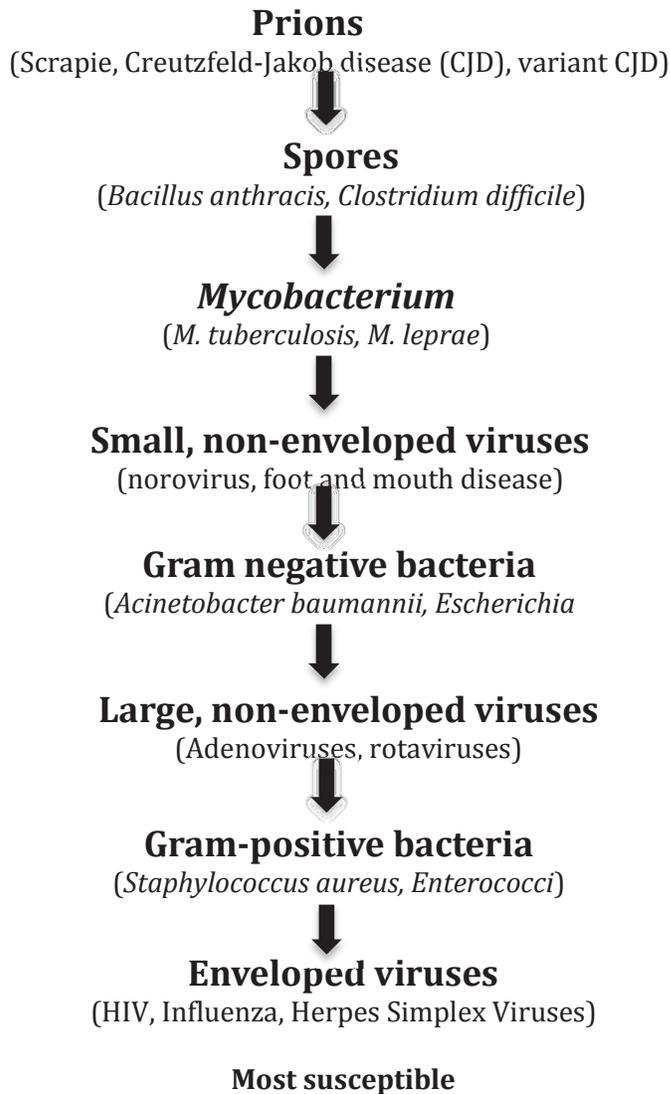


Figure 2: Relative susceptibility of microorganisms to disinfectant activity

Table 1: General characteristics of disinfectant agents

Disinfectant (example)	How it works	Effective Against					Contact Time	Major Uses	Advantages	Disadvantages
		Bacteria	Viruses	M.tb*	Spores					
Alcohols, 60-90% (ethanol, isopropanol)	Disrupts cell membrane	+	+/- ¹	-	-	10-30 min.	- Some medical instruments - Skin cleaning	- Good general use disinfectant, no residue - Inexpensive - Compatible with others (Quats, phenols, iodine) - Non-corrosive - No residue - Active in presence of organic matter	- Flammable - <50% solution ineffective - Not active against some viruses or when organic matter present - Evaporates quickly - Toxic - Eye irritant	
Aldehydes, 0.2-8% (glutaraldehyde, formaldehyde)	Denatures proteins & disrupts nucleic acids	+	+	+	+	10-600 min.	- CIDEX used for medical equipment - Generally gaseous for large areas	- Non-corrosive to metals, rubber, plastic and cement - Non-flammable - Broad-spectrum activity - Active in presence of organic matter	- Toxic and carcinogenic - Respiratory, skin & eye irritant - Leaves residue - Works best at pH >7 and high temperatures	
Chlorine Compounds, 500-5000 ppm (Clorox Bleach)	Denatures proteins & enzymes Disrupts cell membrane	+	+	+	+	5-30 min.	- Hospital, ambulance, household surface cleaning	- Non-flammable - Broad-spectrum activity	- Toxic - Corrosive - Leaves residue - Skin & eye irritant - Inactivated by organic matter	

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Disinfectant (example)	How it works	Effective Against				Contact Time	Major Uses	Advantages	Disadvantages
		Bacteria	Viruses	M.tb*	Spores				
Iodophors, 25-1600ppm (Providone)	Disrupts proteins & DNA synthesis	+	+	-	-	10-30 min.	- Skin antiseptic	- Non-flammable - Considered relatively safe - Rapid action - Active in hard water - Can be used for food prep surfaces	- Toxic if ingested - Corrosive - Leaves residue and can stain skin/material - Respiratory, skin & eye irritant - Inactivated by organic matter and QACs
Oxidizing Agents, 0.2-6% (Peracetic Acid)	Denatures proteins & lipids Disrupts cell membrane	+	+	+	+	5-30 min.	- Medical, surgical & dental equipment - Surfaces - Water treatment	- Sporicidal - Safe breakdown products (biodegradable) - Non-flammable, non-toxic - Rapid action - Broad-spectrum action - Active in presence of organic matter and at cold temperatures - No residue	- Toxic in concentrate - Skin & eye irritant
Phenolic Compounds, 1-5% (Pine Sol)	Disrupts cell membrane	+	+/-	+	-	10-30 min.	- Floor and surface disinfection	- Non-flammable - Active in presence of organic material and hard water - Stable and biodegradable	- Corrosive - Leaves residue - Skin & eye irritant
Quaternary Ammonium Compounds (QACs) (Roccal or Zepharin)	Disrupts cell membrane	+	+/-	-	-	10-30 min.	- Some medical equipment and surfaces	- Non-corrosive, non-flammable - No residue	- Toxic - pH dependent (>3.5) - Inactivated by organic matter, soap, hard water & detergents - Skin & eye irritant

**Mycobacterium tuberculosis*

¹Variable results dependent on virus identity

2.5 Disinfectant Selection

The selection of an appropriate chemical disinfectant depends on many factors including, but not limited to, safety and handling issues, environmental toxicity, microbial resistance, materials compatibility, and spectrum of activity (Table 2).

Of importance to those responsible for the disinfection process, safety and handling issues arise, as microbicides in general must be handled with caution. Poisoning from ingestion of microbicides with serious health consequences has been reported [16] and occupational exposure can result in immediate immune reactions such as hypersensitivity and contact dermatitis [16]. The mechanism of action for many disinfectants involves direct interaction of the active ingredient(s) with nucleic acids (DNA) and/or proteins (including enzymes). Therefore, they possess the potential to affect DNA replication in a number of ways, including mutagenesis [17]. Several microbicides are recognized as carcinogens, for example formaldehyde [18]. Safer microbicides are desirable, but proper training for handling is also necessary.

Environmental safety of disinfection waste products is of concern because most microbicides also contain detergents and inert ingredients that may potentiate their action and toxicity, but this has not been systematically examined. It can take weeks or months for microbicides to break down in the environment and the formation of disinfection by-products (DBPs) occurs that may be mutagens or carcinogens for example from chlorine [19].

Sub-lethal exposure of microorganisms to microbicides perpetuates selection of less susceptible colonies that may lead to increased “tolerance” or “resistance.” Therefore both the disinfectant and the delivery system must be sure to deliver sufficient “overkill” to minimize the likelihood of sub-lethal exposures.

A desirable attribute for the disinfection process in many arenas, particularly healthcare, is materials compatibility – that disinfection will not affect complex medical equipment. Many microbicides cause corrosion or damage to surfaces that are disinfected regularly. Bleach, for example, is corrosive to metal surfaces and also tends to crack rubber components of equipment.

Lastly, the spectrum of activity is a major concern when selecting the appropriate disinfectant. As demonstrated above, different disinfectants have differing activities on a range of microbes making it necessary to select a microbicide that will meet the required needs.

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Table 2: Key considerations in safe and effective use of disinfectants
(Adapted from Table 3.1, [16])

Factor	Problems	Examples
Toxicological	Occupational handling	- vapours cause respiratory sensitization - skin & eye irritation
Microbiological	Resistance Product storage Production dilution Improper disinfectant used	- pathogens may develop resistance - microorganisms may grow in disinfectants with improper storage - improper dilution can affect efficacy of disinfectant - use of wrong disinfectant can lead to propagation of microbes not killed
Chemical	Improper storage Corrosion Improper mixing	- unstable disinfectants – can lead to fire or explosions - use of improper disinfectant for material can lead to corrosion - mixing incompatible chemicals can cause undesirable chemical reactions (e.g. noxious gas production, neutralization of microbicidal activity)
Environmental	Air quality Water and food quality	- use of gaseous or volatile disinfectants affects air quality - microbicides and breakdown products can contaminate food/water and groundwater
Training	Insufficient or improper training	- improperly trained personnel (preparation, use, storage and disposal of disinfectants) can lead to any of the above situations

3. Commonly Used Disinfection and Decontamination Practices

Current disinfection practices used in hospital and by emergency medical services, including Ottawa's Paramedic Services, are manual – surfaces and equipment are cleaned through wiping by hand (Figure 3), typically with chlorine-based solutions. This method includes scrubbing of floors, walls, equipment and exposed portions of the furniture with disinfectant. However, the practice of disinfection through hand wiping has several limitations. These limitations include the inability of the disinfectant to contact hard-to-reach places, harmful effects caused to humans through contact and inhalation, and the often overlooked, necessary minimum contact time of 10 minutes for kill action of the disinfectant (recommended by the EPA) is hard to achieve, particularly on vertical surfaces.



Figure 3: Manual decontamination (wiping by hand)

Several studies have examined the effectiveness of manual cleaning methods in hospitals and emergency medical service (EMS) vehicles. Nigam *et al.* [20] examined bacterial contamination levels in EMS vehicles in Wales before and after manual cleaning procedures were performed. After cleaning, many sites (almost half) were still contaminated and some sites that were previously uncontaminated were found to be contaminated as a result of cleaning methods. Alves and Bissell [21] reported on the unannounced sampling of 5 areas in 4 EMS vehicles in Maryland. Specimens grew moderate to large amounts of environmental and skin bacteria. Of the 7 species isolated, 4 were substantial causes of HAI and 3 of these 4 species of those found to possess antibiotic resistance patterns [21]. Alarmingly, all of the detected microbes were susceptible to disinfectant agents employed by Maryland EMS. In the hospital setting, surfaces were cultured in the rooms of patients with known VRE or *C. difficile* infections after terminal cleaning. In the VRE positive rooms, 91% of specimens before cleaning and 71% of specimens after cleaning were positive for VRE. In the *C. difficile* rooms, 100% of specimens before cleaning and 78% of specimens after cleaning were positive for *C. difficile* [22]. Patient room cleaning in 36 acute care hospitals in US were fluorescently marked prior to cleaning (fluorescent targeting method) to evaluate the thoroughness of terminal room disinfection of environmental surfaces between patients [23][24]. Only 48% of rooms were adequately cleaned such that the fluorescent markings were removed. Overall, thoroughness of cleaning and disinfection of EMS vehicles and hospital rooms by manual methods is less than desirable.

3.1 Example Paramedic Services Vehicle Decontamination Practices [25][26]

Ottawa Paramedic Service (OPS) maintains emergency vehicles and equipment in a clean and sanitary condition to ensure patient and paramedic safety. Equipment and Supply Technicians are responsible for routine cleaning of emergency vehicles between each patient call or vehicle assignment. This includes cleaning of the crew compartment with disinfectant, germicide, degreaser and glass cleaner as applicable. For example, the cab floor is mopped with Percept (germicide) and Virox (disinfectant in a task wipe) is applied to high use areas such as door handles, controls on the dash, and the steering wheel. Similarly, the patient compartment is emptied of soiled linen/garments and waste. The germicide is used to clean the floor and task wipes are applied to high use areas (bench seat, patch, phone, action wall).

Routine practices applied for interaction with patients include universal precautions such that all patients are considered to have a communicable disease and treated accordingly. Before patient contact a risk assessment for communicable disease is performed using patient history, chief complaint, signs and symptoms to assist in the determination.

For routine cleaning and disinfection of emergency vehicles, Virox multi-purpose cleaning wipes (Diversey, Wisconsin), are utilized. A clean wipe is used to wipe down each surface (top to bottom and clean to dirty). The surface is then allowed to dry for at least 10 minutes. Virox wipes are moistened toilettes that serve as a multi-purpose cleaner and disinfectant that contain activated hydrogen peroxide (AHP) as the active ingredient. Virox kills bacteria before the disinfectant solution dries with a kill time for 0.5% AHP of 30 seconds and a dry time of 2 minutes.

Virox wipe disinfection is the method of choice with a few exceptions. Bleach (1:10 dilution) is used for disinfection in suspected or identified cases of *C. difficile*, tuberculosis, CJD, SARS, AI, or Hemorrhagic fever viruses. Bleach disinfection is performed at headquarters before the emergency vehicle is put back in use due to the need to ventilate fumes and employee safety issues.

In summary, wiping by hand is performed for cleaning and disinfection of OPS emergency vehicles. The efficacy of this practice is limited by reach, time and human error; throughput is by necessity very limited and could be a significant problem in an outbreak or terrorism event.

3.2 Example Farm Decontamination Practices [27][28]

CFIA is responsible for controlling viral and bacterial outbreaks among animals (livestock) in Canada. The disease control steps include the humane depopulation (destruction) of affected animals, disposal of the carcasses and thorough cleaning and disinfecting (C & D) of all barns, buildings, equipment, vehicles and materials associated with animal care on the infected premises. Proper C & D ensures the microbe is inactivated to eliminate the risk of disease transmission. The following description of the C & D process is a generic standard operating procedure (SOP) that is used to develop a farm specific SOP that is reviewed and approved by CFIA.

Following animal depopulation, manure piles must be treated. This is accomplished by a bio-heating process, or composting, during which the temperature of the manure pile is monitored. The temperature must reach the killing point for the microbe that is responsible for the outbreak. C & D of the barn and equipment then proceeds in two stages: dry cleaning and then wet cleaning.

Dry cleaning involves the removal of all dry, organic material from all surfaces of the barn and equipment. This is accomplished by a combination of scraping and sweeping; and/or using a foaming

agent (used to cover dust in the barn to reduce the amount of particulate in the air); and/or blowing down the barn and equipment with high pressure air. This process includes the feeding, ventilation and lighting systems within the barn. For equipment and vehicles the process entails C & D of the body of vehicles (all sides and surfaces), wheel wells, inside of tires, under the carriage, under/behind bumpers, grill, fan and radiators, and the interior of the cab if necessary.

Once dry cleaning is completed, wet cleaning can proceed. Wet cleaning consists of first spraying the barn and equipment with a cleaning solution, scrubbing and then rinsing them with water under low pressure. Wet cleaning incorporates the following: preparation of suitable disinfectant according to the label directions; covering of all electrical components and outlets; application of the cleaning solution to all surfaces of equipment and building structures by spraying, with a sponge/mop, or by immersing the object in the solution; allowing the solution to remain in contact with surfaces for the recommended time for the disinfectant (stated on label); scraping and scrubbing areas and applying additional cleaner as necessary; and rinsing according to manufacturer's directions (if necessary).

Throughout these processes staff must be kept safe while minimizing the potential for the spread of the microbe from 'hot zones' (where cleaning is occurring) to 'cold zones' (designated areas free of contamination). Staff wear PPE during C & D for this purpose. PPE worn includes hair nets, rubber gloves, goggles or face shields, face masks (NIOSH approved), disposable hooded coveralls (e.g. Tyvek suits), rubber boots or disposable protective covers, and using duct tape to secure legs/sleeves. This process also involves the use of checklists to ensure proper donning and doffing of PPE because of the ease with which contamination can occur during these processes.

3.3 Example Food Processing Facility Practices [29]

Food facilities typically manually wash as many surfaces as possible with an anti-microbial solution in an attempt to kill as many as possible contaminating microorganisms. The type of disinfectant used and the frequency of disinfection vary by facility.

Some microorganisms typically survive the process either because the agent did not reach them at the proper concentration for the required contact time (e.g. deep within food processing machinery), or because the microorganisms have developed mechanisms to cope with some cleaning agents and temperatures. Further disinfection efforts may fail if biofilms, colonies of microorganisms that hold together on surfaces, have formed in the environment. Biofilms occur widely in nature and may become major problems in wounds and surgical instruments as well as in foods and processing facilities. Biofilm prevention is a major issue in food processing and biofilm removal even more important should prevention fail.

Terminal cleaning practices involve routine (e.g. monthly) thorough cleaning of a facility including all surfaces and equipment. Some dry facilities do not perform terminal cleaning processes. If microorganisms are not being completely removed, they can slowly build up their population and spread over larger areas making the chances of a contamination much higher.

3.4 Example Bioterrorism and Military Response Practices

The current procedures followed by the Canadian Forces (CF) response teams for handling a biological threat in the environment are to establish hot and cold zones, decontaminate and quarantine. In the area between the hot and cold zones a decontamination tent is set up. Within the tent is a shower for cleaning exposed individuals who are then quarantined for a defined period to ensure that they are free from infection. Any equipment used in the hot zone (e.g. detectors and radios) must either be bagged (e.g.

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Ziploc bag around the radio) – their use being limited when bagged in this fashion – or the equipment must be washed down with decontaminating solution by hand. The latter option results in weathering/breakdown of the equipment that must then be destroyed.

Military personnel on-scene must also be protected from the biological hazard. Personal protective equipment similar to that worn by CFIA agents is worn for this purpose. PPE worn includes rubber gloves, goggles or face shields, face masks, Tyvek suits, and boots. The donning and doffing procedures for PPE pose a contamination risk.

One relevant method of disinfection adopted by the military is a heavy duty, self-contained, mobile CBRN (Chemical, Biological, Radiological, Nuclear) decontamination system that delivers CASCAD (Canadian Aqueous System for Chemical/Biological Agent Decontamination) foam. The CASCAD foam was developed for military chemical, biological and radioactive agents. The dispensing system has the capability to decontaminate approximately 10,000 square metres per hour, and CASCAD foam is effective in only 30 minutes, proven to destroy generally >7 log of *B. anthracis* and other biological agents [30]. CASCAD foam can also be delivered using a man- portable backpack and Dolly Air Foam System (Figure 4), which uses air pressure to force CASCAD decontaminant and produce thick, sticky foam to kill biological agents. There are a number of limitations for the use of CASCAD foam for general purpose large area decontamination:

- i. the mobile unit suitable for delivery of large volumes weighs approximately 3600 kg;
- ii. after use of the decontaminant, the foam needs to be removed and the area cleaned for re-use; and
- iii. CASCAD foam is not compatible with sensitive equipment and generally destroys whatever it decontaminates unless it is composed of a hardened material.

Identified areas where improvement could occur include decontamination of sensitive equipment without resulting in its destruction, safer doffing practices for PPE, and more portable systems for large area decontamination.



Figure 4: Dolly Air Foam Backpack System for CASCAD [31]

3.5 Decontamination of Large Spaces using Airborne Delivery of Disinfectant

Large space decontamination is troublesome because not all surfaces can realistically be sprayed and wiped. Decontamination of large spaces involves exposing a confined space to a gas, vapour or aerosol with sporicidal activity to eradicate, or sufficiently reduce, a known bioburden. Space decontamination may also be performed as a preventative measure as part of a cleaning cycle to ensure a space is free of contaminants. The space undergoing decontamination can be as small as a laboratory biological hood or as large as an entire building. Some examples include manufacturing rooms, laboratories, buildings, containment suites and the equipment contained within those spaces (e.g. refrigerators or computers).

There are several practices that distribute disinfectants into the air to achieve disinfection of large spaces including formaldehyde steam, gaseous chlorine dioxide, vaporized hydrogen dioxide and recently dry fogging. These methods distribute the disinfectant in an effort to achieve 100% coverage of an enclosed space, either as a gas/vapour, or as an aerosol. The following sections will review existing and emerging technologies that use airborne chemicals to decontaminate.

These methods for large space decontamination are compared in Table 3. Significant disadvantages of a given technology relative to others are noted in red.

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Table 3: Large area decontamination technologies

	Formaldehyde Steam (FS)	Gaseous Chlorine Dioxide (GCD)	Vaporized Hydrogen Peroxide (VHP)	Dry Fogging Systems (DFS; Peracetic Acid +/- hydrogen peroxide)
Health Risk	Carcinogenic	Non-carcinogenic	Non-carcinogenic	Non-carcinogenic
Humidity Requirement	65-80%	65-80%	10-60%	Wide range; ≤50% @ 20° C
Microbicidal Activity	Broad	Broad	Broad	Broad
Sporicidal	Yes	Yes	Yes	Yes
Neutralization Requirement	Yes	Yes	No, ventilate	No, ventilate
By Products	Hexamethylene-tetramine (powder)	Chlorites & Chlorates	Water, oxygen	Water, oxygen, acetic acid
Waste generation	Powder	Liquid waste	No	No
EPA Registration	No	Yes	Yes	Yes
Electronics Compatibility	No	No	Yes	Yes
Time for Room Decontamination	Long (12 h.)	Long (12 h.)	1 h.	30 min.
Aeration Time	4-5 h.	<30 min.	4-5 h.	10-30 min.
Cost	\$	\$\$\$	\$\$\$\$	\$\$

3.5.1 Formaldehyde Steam

Formaldehyde steam (FS) has been used for a long time; it was used over 40 years ago in the United Kingdom to decontaminate Gruinard Island of anthrax spores [18]. Formaldehyde is a broad-spectrum sterilant that is sporicidal. FS process is industry accepted and validated. However, there are handling issues and health concerns associated with the use of formaldehyde in a gaseous form, as it is a carcinogen.

Formaldehyde steam is generated by heating paraformaldehyde to 70-80° C and relative humidity must be above 60%. Typically the target concentration is 10,000 ppm (~11 g/m³). To achieve sterilization of a space, conditions must be maintained for 12 hours to achieve decontamination. This is accomplished by sealing off an area (room or tent) in which electric hot plates containing paraformaldehyde are placed. To maintain relative humidity water can be misted into the area, as was done to fumigate the US postal center after the anthrax attack in 2001. The toxic gas is then neutralized by ammonia gas. The result is the formation of a white precipitate or residue, hexamethylene tetramine, which is then washed down with water.

3.5.2 Gaseous Chlorine Dioxide

Gaseous chlorine dioxide gas (GCD) was also utilized to decontaminate several buildings following the anthrax attacks, and has been utilized in the food industry. GCD is a broad-spectrum sterilant that has sporicidal activity at low concentrations (100-1800 ppm). The mechanism of sporicidal activity of GCD is

via oxidation by which it attacks the target cell membrane, and it does not produce any chlorine by-products. GCD is able to penetrate some materials (e.g. porous materials, plastic and rubber).

There are several target conditions required to achieve decontamination, including: air concentration of 750 ppm ClO_2 for 12 hours, with a temperature $> 22^\circ \text{C}$ and relative humidity above 65%. These conditions can be difficult to maintain. The cycle phases of GCD include humidification, decontamination and aeration. A cycle typically takes 3-7 hours. Because GCD is heavier than air, fans are required for uniform gas distribution in an enclosed space. In addition, to neutralize GCD the exhaust must be passed through sodium sulfite/bisulphite scrubbers and a carbon bed to prevent leakage of toxic ClO_2 gas into the environment. This generates a large volume of liquid waste.

3.5.3 Vaporized Hydrogen Peroxide

Vaporized hydrogen peroxide (VHP) systems were developed in the early 1990s and have been used for over a decade in medical, biological and pharmaceutical industries. There are a number of commercially available VHP automated systems available for disinfection of equipment and rooms. VHP activity is broad-spectrum and it is sporicidal at low concentrations (150-700 ppm). Neither VHP nor its breakdown products, water and oxygen, are toxic. Thus VHP is a safer option than FS or GCD and it does not leave a residue.

VHP systems involve heating 30-35% hydrogen peroxide and pushing it through a vaporizer (dispersal mechanism) to distribute the vapour into the air. The cycle phases of the VHP process include dehumidification, conditioning, decontamination and aeration.

To maintain vapour concentrations, ambient temperature and $<50\%$ relative humidity must be maintained and monitored throughout the decontamination phase. Cold surfaces can increase condensation and affect vapour concentrations. A typical cycle time is 45 minutes to an hour for a target dose of 250-500 ppm and the disinfection of a room. However, because there are no toxic products the room can simply be aired out and it does not require a neutralization process. The VHP decontamination process generally takes 4-7 hours (including airing out the room).

VHP is largely compatible with materials except nylons and galvanized aluminum with long exposure times. However, VHP is absorbed into plastics and materials, which extends aeration times. Rooms with a lot of equipment can also pose a problem, particularly if the equipment affects the air temperature around it.

3.5.4 Peracetic Acid and Dry Fogging

Peracetic acid, or peroxyacetic acid (PAA), was introduced as a bactericidal agent in the 1950s. The broad spectrum of activity of PAA is chief among its many positive attributes that include biodegradability, activity in the presence of organic matter and under a range of environmental conditions. PAA is used in health care for sterilization of medical equipment (e.g. endoscopes) and in the food industry for disinfecting sewage sludge.

PAA is a strong oxidant with rapid, broad-spectrum activity; it is a clear, colorless liquid with no foaming capability and has a strong pungent vinegary odour. As an acid it has a pH of less than 2 and is soluble in water and polar organic solvents. PAA's killing activity relies on the release of active oxygen. PAA disrupts the cell membrane of target organisms and causes cell lysis [17]. In addition, PAA also denatures proteins, possibly explaining its sporicidal activity. The broad-spectrum of activity of PAA is attributed to its multiple targets of activity.

PAA is a more potent microbicide than hydrogen peroxide (H_2O_2), another commonly used peroxy-disinfectant, being rapidly active at low concentrations against a wide spectrum of microorganisms [32]. Even in the vapour phase, PAA demonstrated the highest kill rate at the lowest concentration [32]; H_2O_2 required much larger doses than PAA for the same level of disinfection in water [33]. The ranking of PAA efficiency against microorganism can be generalized to: bacteria > viruses > bacterial spores > protozoan cysts [35]. PAA maintains its antimicrobial action at low temperatures and is relatively active in the presence of organic matter.

The decomposition products of PAA are oxygen, water, and acetic acid, the latter product being responsible for the strong vinegary odour associated with PAA. Dilute PAA solutions are safe and are compatible with a range of materials. Pure aluminum, stainless steel, and tin-plated iron are resistant to PAA; but plain steel, galvanized iron, copper, brass and bronze are susceptible to reaction and corrosion. As a dry fog the range of material compatibility is likely to be increased because of the absence of water on contact with the surface. However, the stability of dilute PAA solutions (<5%) is short (i.e. the solution starts to lose its activity quickly) compared with more concentrated solutions (40%). Because of their instability at low concentrations, PAA solutions are generally stored in concentrated form and diluted prior to use. In comparison to H_2O_2 , PAA is less stable: a 40% PAA solution loses 1-2% activity per month, while a 30-90% H_2O_2 solution only loses <1% per year.

The large antimicrobial spectrum, short exposure time, and non-toxic decomposition products make PAA a suitable disinfectant for many industries. Not surprisingly, PAA has been used widely as a liquid disinfectant and sterilant in food processing, beverage, medical, and pharmaceutical industries. It has also shown considerable potential when used in dry fogging systems, as described below.

Dry Fogging Systems (DFS) utilize an ultrasonicator or aerosolizer to form very small (1-10 μm) particles of disinfectant that are rapidly dispersed into the air. The result is the creation of a dry fog of disinfectant that both remains airborne to act upon any airborne biological contamination, as well as coating surfaces to act upon deposited contaminants. After an appropriate duration, the fog in the air is evacuated by vacuum or HVAC systems, while the disinfectant continues to act upon surfaces. These systems typically utilize a peracetic acid solution (0.5-6%, with or without hydrogen peroxide and halide ions)² and claim a number of benefits over manual cleaning methods.

“Fogging” as a general term refers to the dispersal of finely disposed droplets of disinfectant within a room (Figure 5). There are two types of fogging: wet fogging and dry fogging. Wet fogging is the process of taking a liquid disinfectant and dispersing it into the air in the form of fine micro-particles. However, at the larger size generated (>10 μm), the droplets fall out of air faster, and their large size leads to bursting and the object it lands on becomes wet. This can lead to problems with electronics and other sensitive equipment.

On the other hand, “dry” fogging is defined as generating an aerosol particle of less than 10 μm in size. Due to their light weight, the particles distribute more evenly throughout a volume and are carried easily by air currents and diffusion. In addition the water solvent evaporates quickly into the air, with all surfaces left dry after the application.

This method offers several potential advantages above manual cleaning and disinfection practices. These portable systems release controlled and consistent amounts of disinfectant into the area, removing the element of human error. Disinfectant reach is extended because the fog penetrates inaccessible areas (hard to reach places) and covers virtually every surface. Turnaround time is improved because the delivery

² Although there are other solutions that can be dispersed and will be discussed later.

systems are much more rapid than manual methods, while the requirement for manual labour is eliminated. Surfaces are not wetted, and residual airborne fog is removed by evacuation of room air.

DFS can employ solutions containing peracetic acid that is, as noted above, effective against a broad spectrum of microbes, is non-corrosive, non-toxic and environmentally safe, and can be combined with other disinfectant agents to improve the spectrum of activity even further. The systems may be effective at low temperatures and with high soil load tolerance.



Figure 5: Dry fogging of a hospital patient room [34]

The major differences between the dry fog systems and vaporized hydrogen peroxide are disinfectant efficacy, coverage, corrosion, portability and cost. The disinfectant used in VHP systems contains ~35% hydrogen peroxide (H_2O_2) or 300-700 parts per million (ppm). This high concentration has a high vapour density and hence stays low in a room. It requires air movement (in a sealed room this requires the use of fans) for the vapour to reach all areas of the room. In addition, this high concentration is also corrosive. In contrast the dry fog systems are mostly peracetic acid and have <75 ppm of H_2O_2 and preliminary studies demonstrate that it is not corrosive. Further, the distribution of dry fog obtains a much greater coverage (claims of 100%). The VHP systems require a constant temperature and humidity level to be maintained during the sterilization process that requires monitoring. This is a problem when portability is considered. DFS companies, on the other hand, purport portability as an attribute of the system afforded by the higher tolerability for temperature and humidity ranges.

3.6 Summary of Current Decontamination Methods

Current cleaning and disinfection practices are mostly manual. Personnel in the respective industry would wipe, mop and apply antimicrobial solutions by hand, with or without personal protective equipment as necessary. These practices are time consuming, subject to human error, fumes can lead to health concerns, hand wiping often leaves hard to reach areas contaminated, they require frequent changes of wipes due to spread of contamination by the cleaning implement, they can be corrosive to equipment, and contamination of PPE and equipment is a concern. Further, these methods often are not sufficient to decontaminate more resistant microorganisms, such as spores.

Various techniques for more automated delivery of vapours, foams, and aerosols exist. Each has advantages and disadvantages. Dry fogging technologies using peracetic acid combined with other

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disinfectants offer a potential system of decontamination that addresses a number of current capability gaps, as discussed in the next section.

4. Dry Fogging Systems

There are several dry fogging systems available on the market, with four available in Canada including: MinnCare® Dry Fog System (19L), MinnCare® Mini Dry Fog System (0.5L), Zimek® Touch-free Rapid Decontamination Systems (ROC/MAX-Micro-Mist Generator and Z-vac Micro-Droplet Evacuator), and Altapure® Ultrasonic Decontamination System (HJ600 System). These systems all employ an Ultrasonication method to generate micro-particles of disinfectant solution that are then dispersed into the air and allowed to persist for a prescribed “dwell” time, the required contact time to kill all affected microorganisms. Zimek and Altapure systems also include evacuation systems that vacuum fog particles out of the air at the conclusion of the dwell time. Both MinnCare systems are designed to incorporate building HVAC systems, some other method of aeration, or require considerably longer periods for fog dispersal since there is no evacuation component of the system (which is reflected in the cost of the unit). All 4 systems market a disinfectant that is effective against spores and contains peracetic acid solution (with or without hydrogen peroxide and/or silver). In addition, the Zimek company markets 2 other disinfectants: Sporicidin, a phenol-based solution; and Vital Oxide, composed of chlorine dioxide in solution with a quaternary salt (DMBAC). The specifics of each system (Table 4) are reviewed below.

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Table 4: Commercial dry fog systems

	Altapure	Mar Cor		Zimek		
		Full Size DFS	Mini DFS			
Particle Size	0.2-2 µm	7.5 µm	7.5 µm	0.3-5 µm		
Disinfectant	4% PAA	0.8% PAA + 1% H ₂ O ₂	0.8% PAA + 1% H ₂ O ₂	Sporicidin	Vital Oxide	Steriplex Ultra
Health Canada Approval	Yes	Yes	Yes	Yes	Pending	N/A ¹
Sporicidal	Yes	Yes	Yes	No	No	Yes
Cycle Time (hospital patient room)	45 min.	45min.	45min.	60 min.		
Room Size Ability	*30,000- 106,000 ft ³ (850-3,000 m ³)	35,000 ft ³ (1,000 m ³)	1,100 ft ³ (20 m ³)	75,000 ft ³ (2,124 m ³)		
Compressed Air Supply Requirement	No	75 psi	75 psi	No		
Room evacuation method included	Yes	No	No	Yes		
Cycle Time (hospital patient room)	45 min.	45min.	45min.	60 min.		

*scalable

¹Use as anti-anthrax agent

4.1 Available Dry Fogging Systems

4.1.1 Altapure

The Altapure DFS (Figure 6) is targeted for use in health care facilities for patient room disinfection. The system has the capacity to disinfect an area of 30,000 ft³ in one hour. The system generates very small particles, <5 µm, from liquid disinfectant by means of an ultrasonicator. These small particles are then dispersed through a nozzle into the room. The disinfectant used is a 4% peracetic acid solution that is bactericidal, viricidal, fungicidal and sporicidal. The machine is automated with pre-set cycles for ease of use. A disinfection cycle consists of 3 phases with varying times depending on the size of the room:

- i) Misting (wherein fog is dispersed): 10-15 min.
- ii) Dwell (wherein the fog is allowed to settle): 10-15min.
- iii) Evacuation (wherein the fog is sucked out of the air): 10-15 min.

For a 1,000 m³ room, an entire disinfection cycle would last 45 min.



Figure 6: Altapure Dry Fog System (Model HJ600)

4.1.2 Mar Cor Purification System

The Mar Cor dry fog systems (Figure 7) deliver a vapour of fine particles (7.5 µm in size) of Minncare® Cold Sterilant. This fine vapour is able to penetrate all reaches of a space. The small droplets bounce off solid surfaces to avoid excessive condensation and corrosion associated with larger droplets. The system is adaptable in height, and for the size of the room, with the addition of nozzles to suit any sized room (up

to 35,000 ft³). The Minncare cold sterilant is composed of 22% hydrogen peroxide and 4.5% peracetic acid. The system is hands free (operated by a remote control) and the DFS unit is autoclavable to ensure sterility. The process time is short (<3h depending on the size of the room) but it is dependent upon the ventilation system before readmission to a room is deemed safe (HVAC system aerates the room). The dry fog vapour detection system provides assurance of proper ventilation for re-entry.



Figure 7: MinnCare Dry Fog System (full size; 22 L capacity)

The Mar Cor Mini DFS (Figure 8) offers the same advantages and system basics as the full size model but with the ability to adapt to smaller size restrictions. The Mini Mar Cor model has a 500 mL cold sterilant capacity that is effective at up to 825 ft³ of space. This DFS unit also has a short process time (<1 h depending on size). It is flexible in that it can adapt to space dimensions (e.g. a fume hood or biosafety cabinet) and it is easily transportable.



Figure 8: MinnCare Mini Dry Fog System (500 mL capacity)

4.1.3 Zimek

The Zimek system (Figure 9) uses a fogging Micro-Mist[®] Generator (ROC or MAX) which produces a fine fog of disinfectant that is dispersed throughout the room or vehicle. This fog will penetrate normally inaccessible areas, going wherever free air flows within an enclosed space, resulting in a more thorough disinfection process. The second part of the decontamination is Zimek's Z-vac[®] Micro-Droplet Evacuator, a mobile "clean-room" filter that cleans the air of the fog, at over 56,000 L/minute, to permit rapid reoccupation of a room or vehicle. The two components constitute Zimek's Touch-Free[™] Rapid Decontamination System.

These systems are automated and the decontamination process is completed in three cycles: Misting (dispersion of mist), Dwell (sit time) and Evacuation (removal of fog) phases. The total time for decontamination of a patient room is approximately 1 hour.



Figure 9: Zimek Dry Fog System – Micro-Mist Generator (Right) and Evacuator (Left)

Zimek offers two disinfectant products for routine use with their micro-misting system (Table 5) and a third option specifically designed for biological attack scenarios (anti-anthrax agent). The two routine use agents are Vital Oxide and Sporicidin. Vital Oxide contains chlorine dioxide and a quaternary salt-based solution that kills vegetative bacteria, viruses, mold and fungi. Sporicidin is a phenol solution (1.56% phenol with 0.06% sodium phenate) that is active against vegetative bacteria, viruses, mold, fungi and tuberculosis. Sporicidin is the disinfectant currently available and approved by Health Canada. The name of this agent, Sporicidin, is misleading since it is not sporicidal. However, the sporicidal agent, Steriplex Ultra, is not subject to Health Canada approval as it is not for use as a regular disinfectant in healthcare. It is considered a CBRN decontaminant to neutralize *B. anthracis*. Steriplex Ultra is active when a mixture of 2 solutions is prepared: i) part A, a solution of 0.03% silver; and ii) part B, a solution of 22% hydrogen peroxide and 15% peracetic acid. The shelf life of the mixture is greatly reduced from the stock solutions, from 1 year to 10 days. Steriplex Ultra kills spores in addition to bacteria, viruses, mold, fungi and tuberculosis.

Table 5: Disinfectants for use with Zimek Dry Fog System

Disinfectant	Active Ingredient	Health Canada Approval	Antimicrobial Activity				
			Bacteria	Virus	Fungus	Spores	M.tb ¹
Vital Oxide	Chlorine dioxide 0.2% + alkyl-DMBAC ²	Pending	+	+	+	-	-
Sporicidin	Phenol 1.56% + Sodium phenate 006%	Yes	+	+	+	-	+
Steriplex Ultra	Peracetic Acid 1.3%	NA ³ (<i>B. anthracis</i>)	+	+	+	+	+

¹M.tb = *Mycobacterium tuberculosis*

²DMBAC = dimethyl benzyl ammonium chloride (a QAC)

³Not for regular use; use as anti-anthrax agent

4.2 Evidence of Dry Fogging System Efficacy

Although the various claims of efficacy for DFS are logical and mostly based on laboratory driven data, there are several knowledge gaps and validation studies required before these systems can be employed in the full range of desired applications with relative certainty of efficacy. These knowledge gaps will be addressed below.

In theory, dry fogging systems employing peracetic acid-based disinfectants are far superior to manual cleaning methods. However, the evidence to support this statement is limited. To evaluate the evidence available two key points must be considered: the quality of the evidence and the level of evidence. The quality of evidence incorporates the accepted methodology and how the results are reported. For example, when examining the efficacy of a disinfectant against bacterial spores a reported ‘percent reduction’ is a less reliable measure than the standard reporting unit of ‘log reduction’. In addition, laboratory conditions are not realistic simply because the parameters of an experiment are controlled, so when these methods are applied in real life situations the same results may not be seen. The level of evidence is also of importance because it incorporates the ‘reality’ of an experiment’s conditions.

The level of evidence and study summaries are presented in Table 6.

The hierarchy of evidence based on study design is defined as follows: bench level data < lab level data < simulated field trials < clinical trials < field trials. These terms are defined as follows:

Bench Level Data (I): Refers to studies examining the efficiency of a liquid disinfectant against bacteria without the use of a fogger. For example, a species of bacteria was spread onto two petri dishes with growth media, one with disinfectant present, the other without.

Lab Level Data (II): Refers to studies conducted in the laboratory utilizing a scaled down fogger in a small chamber to simulate fogging of a room.

Simulated Field Trials (III): Refers to the use of a full size fogger in a room or other natural space to simulate practical use situations.

Clinical Trials (IV): Refers to studies of clinical relevance when dry fogging systems have been implemented in the field. For example, an observational trial reporting the number of hospital acquired infections (HAI) in the 12 months preceding DFS use in the hospital and compared with the number of HAIs in 12 months of DFS use.

Field Trials (V): Refers to interventional studies performed that compared existing methods with DFS in a head to head manner. For example, cleaning rooms in one ward of a hospital with the current method while gathering bacterial cultures throughout, and comparing these to cultures obtained from another ward that is using the DFS system. Ideally, the researchers performing the analysis would be blinded, meaning the research would not know which ward treated and which was not.

4.2.1 Zimek

The Zimek fogging system is marketed for healthcare industry uses such as decontamination of patient rooms in a hospital. This system offers two disinfectants for routine use: Sporicidin and Vital Oxide, both of which are bactericidal, viricidal and fungicidal but are not active against spores. Steriplex Ultra is not yet approved for use in Canada but has enhanced biocidal properties.

Vital Oxide, a chlorine dioxide based solution, has been shown effective at the bench level against *E.coli* [36] and in chambers against *P. aeruginosa* and *S. aureus* with demonstrated kill rates >99% [37]. An observational study using Vital Oxide was undertaken by a 100-bed hospital in the Nashville, Texas [38]. The hospital implemented a terminal cleaning procedure in which a Zimek hand-held fogger with Vital Oxide was utilized to clean contaminated patient rooms. When an environmental specimen was found positive for *Acinetobacter*, hospital staff was notified immediately and the terminal cleaning procedure was initiated. The rates of hospital acquired infections for *Acinetobacter* were then monitored for the following year and compared with the rates of HAI in the year preceding the initiation of the terminal cleaning procedure. In the year preceding fogging, there were 13 cases of *Acinetobacter* out of 25,089 patient days (5.2/10,000) and after the fogging procedure was implemented there were only 2 cases of *Acinetobacter* out of 22,704 patients days (0.88/10,000) [38]. The significant reduction in *Acinetobacter* cases suggests that fogging the patient rooms may have improved disinfection procedures by reaching all areas and improving the kill rate. However, the results may have been biased due to the study design. The combination of awareness of a study and the implementation of the new procedure, which would require new training, may have improved cleaning diligence. These data support the efficacy of Vital Oxide, but do not demonstrate the use of Vital Oxide in combination with the full-scale Zimek dry fogging system.

We were not able to find any data in support of the use of Sporicidin.

Zimek's Steriplex Ultra is a combination of hydrogen peroxide, PAA and silver. Silver, having its own bactericidal action, has been shown to enhance the killing activity of PAA [39]. Lab level studies demonstrate the rapid and highly effective action of Steriplex Ultra against *E.coli* and several species of spores (*B. anthracis*, *B. atrophaeus*, *B. subtilis*, and *C. sporogenes*) [36][40]. The US Army conducted tests against *B. anthracis* spores, with and without organic material. After exposure to Steriplex, *B. anthracis* spores were significantly reduced (at least a 4.8 log reduction (LR) in 15 seconds and at least 7.0 LR in 30 seconds), with and without the presence of organic material [41].

4.2.2 Altapure

Like Zimek, the Altapure system is also targeted towards the healthcare industry. The disinfectant used with this system is a 4% peracetic acid solution. There is one published study that demonstrates the effectiveness of Altapure's system. Several hospital rooms inoculated with a bacterial mixture containing

MRSA, VRE, *A. baumannii*, *P. aeruginosa*, and *C. difficile*. Several samples were taken throughout the room (e.g. door handles, surfaces and walls) and the floor before treatment with the Altapure system. Samples were then re-taken for the same areas and the number of bacteria compared. Pre-treatment the bacterial count was $\sim 10^4$ colony forming units (CFU) per litre whereas after treatment the count was found to be below detectable limits [42].

4.2.3 Mar Cor DFS

In contrast to Zimek and Altapure, the Mar Cor dry fogging system has been used extensively by the pharmaceutical industry in manufacturing plants. Minncare's cold sterilant is utilized in the Mar Cor system and consists of a mixture of 4.5% peracetic acid and 22% hydrogen peroxide. In a lab level study, small chambers with samples of different materials (deck wood, carpet, concrete and wallboard) nebulized with bacterial spores (*B. atrophaeus* and *G. stearothermophilus*) were treated with the Mini DFS. Log reductions of 4 and 5, respectively, were demonstrated [43][44]. Gregersen *et al.* [45] demonstrated efficacy of the Mar Cor system at field level. Carriers inoculated with several viruses (Reovirus 3, a mouse parvovirus (MVM) and Avian polyomavirus) were placed in a room and treated with the Mar Cor DFS (60min. dwell time). This experiment was repeated 10 times over 12 months. For Reovirus 3 the log reduction was 9, for the MVM parvovirus the log reduction was 6.4 and for Avian polyomavirus the log reduction was 7.65. Further, there were electronics present in the room during each DFS treatment. These electronics were found to be functional after 10 exposures to DFS [45].

Mar Cor DFS was evaluated in a simulated laboratory as well as in a high-containment laboratory by Krishnan *et al.* [46]. Biological indicator coupons containing *E. coli*, *S. aureus*, *B. atrophaeus* spores, an enveloped virus (*Vesicular stomatitis virus* Indiana serotype) and a non-enveloped virus (*Human adenovirus 5*) with and without a protein mix (organic material). Coupons were placed at locations (floor, ceiling and wall) throughout the spaces and tested for growth following exposure to DFS. All microbial agents were inactivated by DFS within 30 minutes in the absence of protein. All biological agents were inactivated within one hour in the presence of protein, with the exception of *B. atrophaeus* spores which took approximately 18 hours (overnight) for complete inactivation. Further, this group evaluated the effect of DFS on a personal computer that had been exposed to DFS once monthly for 6 months and found that no functional impairment of the computer [46].

Table 6: Review of evidence of efficacy of dry fogging systems

DFS	Agent	Level of Evidence	Experimental Conditions	Organism(s) Tested	Results	Reference
Altapure	4% PAA	III	Hospital rooms inoculated with ~100 CFU/L of bacterial mixture. Samples taken throughout room & floor. DFS treatment (20min. dwell). Re-sampled.	MRSA, VRE, A. baumannii, P. aeruginosa, C. difficile	Baseline: ~104 CFU/L Post-treatment: not detected	[42]
Mar Car	Minnicare Cold Sterilant	II	Small chamber with samples of deckwood, carpet, concrete & wallboard nebulized with bacterial spores on walls/floors. Sampled. DFS Mini (overnight dwell). Re-sampled. Log reductions (LR) calculated.	<i>B. atrophaeus</i> spores <i>G. sterothermophilus</i> spores	Walls: LR 3.56 Floor: LR 3.93 Walls: LR 3.91 Floor: LR 4.67	[43][44]
		II	Carriers inoculated with viruses placed in a room and exposed to DFS (60min. dwell). Repeated 10 times. Log reductions (LR) calculated. *Electronics present and functional after 10 exposures to DFS.	Reovirus 3 (RV3), MVM parvovirus, Avian polyomavirus (APV)	RV3: LR 9 MVM: LR 6.4 APV: LR 7.65	[45]
		III	Laboratory space validated with coupon carriers placed throughout space (floors, ceiling, walls). Biological agent survival evaluated after DFS exposure at 0.5, 1, 2.5, 5, 7 hours and overnight (~18 h).	<i>E.coli</i> , <i>S. aureus</i> , <i>Vesicular stomatitis virus</i> , <i>Human adenovirus 5</i> , <i>B. atrophaeus</i> spores	Without protein: complete inactivation (CI) in 30 min. With protein: CI in 1 h Without: CI in 30 min With: CI in ~18 h	[46]
Zimek	Vital Oxide	II	Carriers inoculated with bacteria placed throughout 3664 ft ³ room. Sampled. Treated with DFS (30 min. dwell). Re-sampled.	<i>S. aureus</i> <i>P. aeruginosa</i>	>99.9% kill rate >99.9% kill rate	[37]

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DFS	Agent	Level of Evidence	Experimental Conditions	Organism(s) Tested	Results	Reference
		I	Lab: bacteria cultures exposed directly to disinfectant and neutralized at pre-set time intervals. Log reduction (LR) calculated. Compared Vital Oxide (VO) with Bleach.	<i>E.coli</i> <i>B. atrophaeus</i> spores	Bleach: 6 LR (30 sec) VO: 6 LR (30 sec) Bleach: 6 LR (2 h) VO: 0 LR (2 h)	[36]
		IV	Comparison of reported hospital acquired cases of Acinetobacter in 100 bed urban hospital for year before vs. the year after initiated cleaning of rooms with Zimek DFS.	<i>Acinetobacter</i> HAI	Year prior: 13 cases Year with DFS: 2 cases	[38]
		I	Lab: Spore suspensions exposed directly to disinfectant and neutralized at pre-set time intervals. LR calculated. Compared Steriplex Ultra with Bleach.	<i>E.coli</i> <i>B. atrophaeus</i> spores	Bleach: 6 LR (30 sec) Ultra: 6 LR (<30 sec) Bleach: 6 LR (2 h) Ultra: 8 LR (1 h)	[36]
Steriplex Ultra		I	Lab: Spore suspensions prepared and exposed to disinfectant directly. Log LR calculated. Compared Steriplex Ultra to bleach and CIDEX (Glutaraldehyde-based product)	<i>B. subtilis</i> spores <i>B. anthracis</i> spores <i>C. sporogenes</i> spores	Bleach: 5LR (3.5 min) Ultra: 5 LR (45 sec) CIDEX: 5 LR (4min) Bleach: 6 LR (1 min) Ultra: 6 LR (15 sec) CIDEX: 5 LR (190min) Bleach: 6 LR (15sec) Ultra: 6 LR (15sec) CIDEX: 3 LR (20min)	[40]
		III	Lab: Spore preparations with and without organic load were exposed to disinfectant for 15 and 30 seconds. LR calculated.	<i>B. anthracis</i> spores <i>B. anthracis</i> spores with organic load	15 sec: ≥ 4.8 LR 30 sec: ≥ 7.3 LR 15 sec: ≥ 5.1 LR 30 sec: ≥ 7.0 LR	[41]

4.3 Potential Applications of Dry Fogging

The potential applications of dry fogging technology are far-reaching and may be utilized in decontamination efforts of all arenas discussed: health care facilities, emergency response vehicles, farms, food production and processing facilities and military response to biological attacks.

4.3.1 Vehicle & Room Decontamination

Emergency vehicles and health care facility rooms are decontaminated by manual methods typically utilizing chlorine based solutions or wipes. There are many limitations of manual disinfection, including: human error (insufficient contact time for proper disinfection); long turn around that limits the health care system's ability to utilize all available resources; insufficient disinfection of hard to reach places, health concerns for personnel (fumes can be toxic); the risk of spreading contamination with cloths/mops; and resulting corrosion of equipment. Dry fogging systems may alleviate all of these problems by eliminating human error and extending reach (controlled release and distribution), reducing turnaround time for rooms and vehicles, improving safety (breakdown products include water, oxygen and acetic acid), and preliminary studies suggest these systems may be compatible with equipment and active in the presence of organic material (e.g. blood or dirt). Further, DFS may prove a reliable means for decontamination of transient equipment such as blood pressure cuffs, intravenous poles and wheelchairs that circulate in hospitals and remain in emergency vehicles. Many of the features of DFS will reduce the likelihood of increased resistance of microbes to disinfectants (for example, as has occurred with *Acinetobacter* species) by providing a more comprehensive disinfection approach that might be effective even on soiled surface and in the presence of biofilms (both of which have contributed to the resistance of *A. baumannii* [48]).

4.3.2 Farm Decontamination

Once the initial dry cleaning of a contaminated barn/warehouse is completed (removal of all organic material), wet cleaning is performed. During the wet cleaning process disinfectant is applied to all surfaces and equipment with a sponge/mop or by immersing objects in disinfectant solution. This process is subject to the same limitations listed above in section 4.3.1.

During both dry and wet decontamination processes personnel must utilize personal protective equipment (hair nets, rubber gloves, goggles or face shields, face masks, hooded coveralls and rubber boots). It has been demonstrated that even with assistance and secondary layers of PPE, transfer of contaminants occurs. There is potential to utilize DFS to decontaminate PPE before doffing, thus preventing transfer.

4.3.3 Food Facilities and Equipment Decontamination

Food facilities typically perform manual cleaning and disinfection similar to the process outlined above, with the type of disinfectant and frequency of disinfection varying by facility; the same limitations occur (4.3.1). Some microbes survive because the agent did not reach them at the proper concentration, they were in hard to reach places, or because of resistance mechanisms in the population of microbe.

Dry fogging systems could potentially eradicate the microorganisms. The efficacy of DFS in the presence of organic matter is of particular relevance here. Routine use of DFS for decontaminating a facility before an issue arises can reduce the chances of a contamination and/or a recall. This could potentially save money, reduce business disruptions, and perhaps save lives.

4.3.4 Bioterrorism

Spore-forming bacteria, such as *B. anthracis*, can be effective biological attack agents as demonstrated by the 2001 anthrax attacks spread via the US postal service. Dry fogging systems employ peracetic acid-based disinfectant solutions that are effective against a broad spectrum of microorganisms, and most importantly, highly effective against spores. The decontamination methods employed during the postal incident included FS and GCD. The limitations of FS include its carcinogenicity, long decontamination time, incompatibility with equipment and the residue left after decontamination. Although non-carcinogenic, these limitations also apply to GCD. The advantages of DFS over FS or GCD include the lack of residue, shorter turnaround time, compatibility with electronics and it has been suggested that it is effective over a wider range of humidity and temperatures.

4.4 Utilization of Dry Fogging on a Broad Scale: Identifying Project Partners, Technology Readiness Level and Knowledge Gaps

Recently, Defence Research and Development Canada's (DRDC) Canadian Safety and Security Program (CSSP) hosted two Decontamination Workshops [1][2]. The participants in the first workshop were scientists involved with decontamination research and use. The participants of the second workshop were first responders who are on the front line using the decontamination methods. The objective of the two meetings was to identify gaps by bringing together people from many organizations that are responsible for and/or directly involved in CBRN decontamination responses. These reports were intended to capture the identified gaps in our ability to respond to CBRN scenarios and to rank those gaps based on need. The reports were also intended to be utilized as a basis for a targeted investment initiative. This was ideal for our goal of building a roadmap for dry fogging technology as a potentially widely applicable decontamination method.

At these meetings we were able to connect with many people with common decontamination goals and identify potential project partners. This list included:

1. Canadian Food Inspection Agency (CFIA) – Farm Decontamination
2. Canadian Food Inspection Agency (CFIA) – Food Industry Decontamination
3. Canadian Forces (CF)
4. Royal Canadian Mounted Police (RCMP) – Forensics
5. Ottawa Paramedic Service (OPS)
6. Public Health Agency of Canada (PHAC)

Members of this list were all interested in being involved in the DFS research project.

Also, as a result of these meetings we were able to identify several areas for which dry fogging technology has the potential to offer a better methodology/technology than current practices or to fill gaps. The high level knowledge gaps identified were as follows:

1. To identify the best decontamination system for a biological attack, specifically for an anthrax release scenario. This would involve comparisons between available methods (CASCAD, GCD, FS, VHP and DFS). This gap incorporates some of the following points.

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2. To identify decontamination systems compatible with the decontamination of sensitive equipment.
3. To assess the use of DFS as a decontamination method applicable to the removal of PPE.
4. To assess the use of DFS in areas between hot and warm zones.
5. To assess the benefit of DFS with respect to waste management (if we can properly decontaminate an area and equipment/materials within, this would directly affect the amount of waste that needs to be incinerated/autoclaved).
6. To assess what decontamination methods would allow collection of physical evidence without affecting the integrity of that evidence.
7. To assess the potential of DFS decontamination in situations with high soil load (e.g. in food production facilities wherein fat, grease and by-products interfere with decontamination).
8. To assess the potential of DFS decontamination in situations of low/high humidity (e.g. military use overseas in the desert or in tropical climates and CFIA use throughout Canadian seasons).
9. To assess the potential of DFS decontamination in situations of low temperature wherein most disinfectants are rendered ineffective (e.g. CFIA use at a farm outbreak during winter months).
10. To assess the potential for DFS for decontamination of transient equipment within hospitals (e.g. IV poles, blood pressure cuffs, wheelchairs, etc.)

Further, the above gaps identified were applicable to several stakeholders. For example, using DFS to eliminate the risk of contamination while doffing PPE would be applicable both to CFIA personnel during decontamination of farms after an outbreak, and to military personnel and first responders responding to a CBRNE event.

Figure 10 outlines the areas in which dry fog technology may be beneficial and identifies the knowledge gaps that need to be addressed before field studies can be conducted.

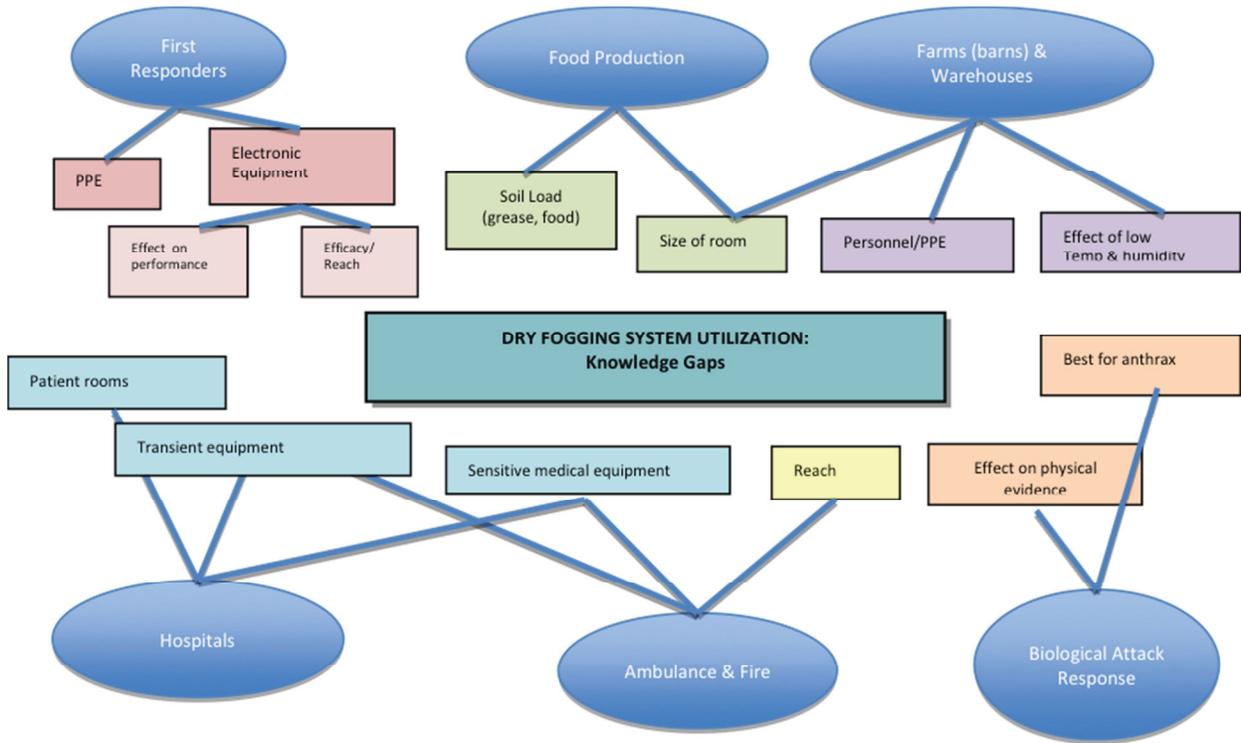


Figure 10: Knowledge Gaps to be filled before Field Operationalization of DFS

5. Study Technical Objectives

In order to operationalize dry fog technology in any of the arenas identified above, there are several technical knowledge gaps that need to be addressed in the laboratory environment. The current technology readiness level (TRL) of DFS is estimated to be between 4 and 7. Proof-of-concept trials have been successfully completed and laboratory studies support DFS use, but the full range of use has not been demonstrated. Further studies are required to bring DFS to above TRL 4.

One of the main findings from previous investigations is the difference in effectiveness of peracetic acid solutions (with or without hydrogen peroxide and silver) on different surface types, e.g. wood or plastic [47]. One of the common uses for DFS identified by different project partners is for decontamination of PPE prior to doffing. However, we first need to determine the effectiveness of PAA fogging on PPE material. Other materials would be tested in parallel including wood, plastic, metal, rubber and porous surfaces because these represent the components of any field equipment of concern (such as radios or detectors). We will also test the working conditions of relevant electronic equipment, such as radios and detectors, before and after exposure to DFS. Because *B. anthracis* spores are the most likely choice for a biological attack and because the spores offer the most robust microorganism and thus the hardest to kill, a simulant (*Bacillus atrophaeus*), was selected as the organism to test DFS against in all experiments. Further, it has been demonstrated that *B. atrophaeus* spores are more resistant to bleach disinfection than *B. anthracis*, *Clostridium difficile* and other *Bacillus* and *Clostridium* spores [49][50]. Temperature ranges (-20°C, -10°C, 4°C, 22°C, 30°C, 50°C) and relative humidity (high, medium and low) are important considerations to be able to operationalize DFS technology, particularly for our Canadian Forces and Canadian Food Inspection Agency partners (based on NATO climate classifications for Canada and the middle east). Soil load will also be investigated to determine the kill rate of DFS in the presence of dirt, grease and food particles. Lastly, we will determine if DFS is effective in hard to reach places such as far corners of a room, vertical surfaces and inside equipment. The series of experiments is outlined in Table 7.

Table 7: Laboratory study parameters

Characteristic	Parameters
Material	Wood, plastic, metal, PPE, rubber, concrete, textiles and polymers
Temperature	-20°C, -10°C, 4°C, 22°C, 30°C, 50°C
Humidity	Low (30%), medium (60%) and high (90%)
Soil load	Dirt, grease, food particles
Equipment Compatibility	Functionality of equipment post exposure to DFS ('n' rounds)
Hard to Reach places	Corners, vertical surfaces, inside equipment, etc.

Prior to the commencement of the above studies, initial qualification experiments will need to be performed to establish the standard operating procedures (i.e. the most appropriate experimental conditions) including, the concentration of *B. atrophaeus* spores and their dissemination, the selection of other biological organisms as efficacy controls, the test conditions of the fogging agents, and sampling procedures to be utilized (method and consistency of recovery).

We estimate the qualification experiments will require approximately one year to complete and the subsequent laboratory experiments 2-3 years. Thus, it will be 3-4 years before field experiments will commence. The proposed roadmap for the project is outlined in Figure 11.

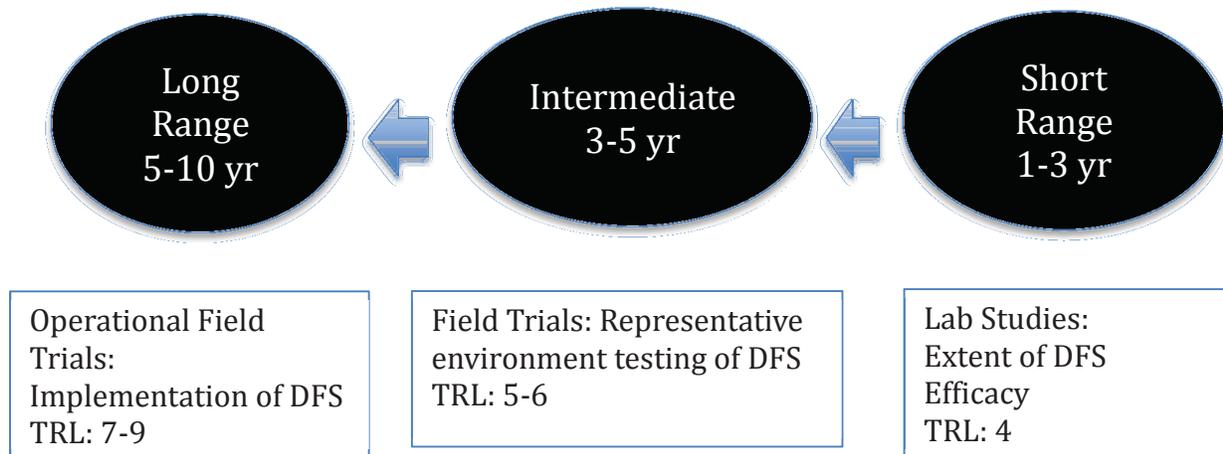


Figure 11: High level roadmap for operationalization of DFS technology

Future knowledge gaps to be addressed during the fielding phase of experimentation include the following:

1. Do laboratory results translate to field conditions?
2. Is the integrity of personal protective equipment affected by DFS agents?
3. Is skin exposure to DFS agents safe?
4. Does DFS treatment inactivate vapour protection (charcoal layer) of PPE?
5. Is the DFS agent effective against all microorganisms (e.g. viruses and resistant bacteria)?
6. Is combination fogging effective (i.e. multiple DFS agents used in succession)?
7. Is there interaction between disinfectants (e.g. Vital oxide and Steriplex)?
8. Does the application of combinations of disinfectants improve efficacy and/or allow for shorter application times?
9. Are these disinfectants effective against chemical agents?
10. What is the best method for DFS deployment in the field? For example, what are the electrical requirements? Is it possible to manufacture a portable, backpack version of a DFS?
11. How does DFS compare with competing technologies (e.g. CASCAD)?
12. Does DFS destroy physical and/or biological evidence (e.g. fingerprints and DNA) at the scene?
13. Does a cost analysis support the use of DFS?

6. Conclusions

The outcomes of two Decontamination Workshops hosted by CSS demonstrated the need for new technology to combat biological threats, including those encountered routinely in the healthcare and food industries. Current cleaning and disinfection methods are not sufficient and several specific needs were identified that cross industries. Across these fields the needs included a technology that would be safe, portable, compatible with equipment and PPE, and effective in the presence of an organic soil load and over a range of temperature and humidity. Dry fogging systems offer a potential solution to this range of issues. DFS may be a robust, safe, rapid, portable technology that has a broad spectrum of activity against biological agents. However, the paucity of published, controlled studies at many levels of evidence makes an assessment of these systems difficult. A thorough investigation of DFS applicability is warranted.

This document reviewed the knowledge gaps surrounding DFS (Figure 13). There is potential for DFS to meet the needs identified across industries. RMCC will work in collaboration with OPS, CFIA, CF, PHAC and RCMP to determine the efficacy of DFS in conditions faced in these fields. The study objectives include assessing the efficacy of DFS with respect to material, temperature, humidity, soil load, compatibility with equipment and reach (Table 7). This collaborative effort will attempt to address these issues and fill the knowledge gaps, towards the goal of operationalization of DFS.

References

- [1] Science DCfS. CSSP Decontamination Science Workshop, October 10-11, 2012, Summary Report. Ottawa: Government of Canada, 2012.
- [2] Research IS. CSSP First Responder Decontamination Workshop Summary. Ottawa, 15 November 2012: ISR Report 13017-01.
- [3] Inweregbu KJ, D.; Pittard A. Nosocomial Infections. *Crit care & pain*. 2005;5(1):4.
- [4] Sunenshine RH, Wright MO, Maragakis LL, Harris AD, Song X, Hebden J, *et al*. Multidrug-resistant Acinetobacter infection mortality rate and length of hospitalization. *Emerg Infect Dis*. 2007;13(1):97-103.
- [5] Peleg AY, Seifert H, Paterson DL. Acinetobacter baumannii: emergence of a successful pathogen. *Clin Microbiol Rev*. 2008;21(3):538-82.
- [6] Falagas ME, Karveli EA. The changing global epidemiology of Acinetobacter baumannii infections: a development with major public health implications. *Clin Microbiol Infect*. 2007;13(2):117-9.
- [7] Villegas MV, Hartstein AI. Acinetobacter outbreaks, 1977-2000. *Infect Control Hosp Epidemiol*. 2003;24(4):284-95.
- [8] Kim J, Smathers SA, Prasad P, Leckerman KH, Coffin S, Zaoutis T. Epidemiological features of Clostridium difficile-associated disease among inpatients at children's hospitals in the United States, 2001-2006. *Pediatrics*. 2008;122(6):1266-70.
- [9] Sivapalasingam S, Friedman CR, Cohen L, Tauxe RV. Fresh produce: a growing cause of outbreaks of food-borne illness in the United States, 1973 through 1997. *J Food Prot*. 2004;67(10):2342-53.
- [10] Prevention CfDcA. Bioterrorism Overview. Atlanta: United States of America Government; 2007 [accessed 2013 February 2].
- [11] Inglesby TV, O'Toole T, Henderson DA, Bartlett JG, Ascher MS, Eitzen E, *et al*. Anthrax as a biological weapon, 2002: updated recommendations for management. *Jama*. 2002;287(17):2236-52.
- [12] Schmitt K, Zacchia NA. Total decontamination cost of the anthrax letter attacks. *Biosecure Bioterror*. 2012;10(1):98-107.
- [13] Peters CJ, Hartley DM. Anthrax inhalation and lethal human infection. *Lancet*. 2002;23;359(9307):710-1.
- [14] Schmid-Hempel P, Frank SA. Pathogenesis, virulence, and infective dose. *PLoS Pathog*. 2007;3(10):1372-3.
- [15] Brazis AR, Leslie JE, Kabler PW, Woodward RL: The inactivation of spores of Bacillus globigii and Bacillus anthracis by free available chlorine. *Appl Microbiol* 1958; 6:338-342.

UNCLASSIFIED

- [16] Sattar SAS, Springthorpe S. The need for safer and better microbicides for infection control. In: Manivannan G (ed.). *Disinfection and decontamination: principles, applications and related issues*. Boca Raton, FL: CRC Press; 2006. pp. 41-58.
- [17] Denyer SPS, Stewart G. Mechanisms of action of disinfectants. *International biodeterioration & biodegradation*. 1998;41:261-268.
- [18] Manchee RJ, Broster MG, Stagg AJ, Hibbs SE. Formaldehyde solution effectively inactivates spores of *Bacillus anthracis* on the Scottish island of Gruinard. *Appl Environ Microbiol*. 1994;60(11):4167-71.
- [19] Richardson SD, Postigo C. Drinking water disinfection by-products. In: Barceló D (ed.) *Emerging Organic Contaminants and Human Health*. Handbook of Env. Chem 2012; 20:93-138. Berlin, Germany: Springer-Verlag.
- [20] Nigam Y, Cutter J. A preliminary investigation into bacterial contamination of Welsh emergency ambulances. *Emerg Med J*. 2003;20(5):479-82.
- [21] Alves DW, Bissell RA. Bacterial pathogens in ambulances: results of unannounced sample collection. *Prehosp Emerg Care*. 2008;12(2):218-24.
- [22] Eckstein BC, Adams DA, Eckstein EC, Rao A, Sethi AK, Yadavalli GK, *et al*. Reduction of *Clostridium Difficile* and vancomycin-resistant *Enterococcus* contamination of environmental surfaces after an intervention to improve cleaning methods. *BMC Infect Dis*. 2007;7:61.
- [23] Carling PC, Parry MM, Rupp ME, Po JL, Dick B, Von Beheren S. Improving cleaning of the environment surrounding patients in 36 acute care hospitals. *Infect Control Hosp Epidemiol*. 2008;29(11):1035-41.
- [24] Carling PC, Parry MF, Bruno-Murtha LA, Dick B. Improving environmental hygiene in 27 intensive care units to decrease multidrug-resistant bacterial transmission. *Crit Care Med*. 2010;38(4):1054-9.
- [25] Ottawa Paramedic Service. Vehicle and Equipment Checks (Processing). Technical Services 2008. p.1-23.
- [26] Ottawa Paramedic Service. Prehospital Infection Control (PIC) Document. [Routine Practices]. 2008.
- [27] Canadian Food Infection Agency. Cleaning and Disinfecting Broiler Barns after a Notifiable Avian Influenza (H5 or H7) Outbreak. Generic Standard Operating Procedures and Checklists. Nepean, Ontario: CFIA; 2008.
- [28] Canadian Food Infection Agency. Vehicle and Large Equipment C&D Checklist. 2012. p. 1-4.
- [29] Agriculture HSPD-Fa. Federal Food and Agriculture Decontamination and Disposal Roles and Responsibilities. Washington: United States Department of Health & Human Services, USDA; 2005 Contract No.: HSPD-9.
- [30] Calfee MW. Biological Agent Decontamination Technology Testing: Technology Evaluation Report. Environmental Protection Agency. EPA/600/R-10/087. September 2010.

UNCLASSIFIED

- [31] Allen-Vanguard. <http://reports.hms-online.org/ViewProduct.aspx?CategoryId=175&ProductId=711>. Accessed March 2013.
- [32] Baldry MG. The bactericidal, fungicidal and sporicidal properties of hydrogen peroxide and peracetic acid. *J Appl Bacteriol.* 1983;54(3):417-23.
- [33] Wagner M, Brumelis D, Gehr R. Disinfection of wastewater by hydrogen peroxide or peracetic acid: development of procedures for measurement of residual disinfectant and application to a physicochemically treated municipal effluent. *Water Environ Res.* 2002;74(1):33-50.
- [34] Zimek. http://www.zimek.com/img/ZimekTech_Healthcare-Demo.jpg. Accessed March 2013.
- [35] Kitis M. Disinfection of wastewater with peracetic acid: a review. *Environ Int.* 2004;30(1):47-55.
- [36] Kimball SM, T; Phillips, S; Bissegger, S; Bodurtha, P; Weber, K; Dickson, E. Sporicidal activity of Bleach and Steriplex Ultra. Unpublished results.
- [37] Jeske AS. Evaluation of a room decontamination system. Eagan, MN: ATS Labs. 2010 Contract No.: A09366.
- [38] Hulette RB. An intervention to reduce the rate of hospital-acquired *Acinetobacter* infections in an urban community hospital. [Poster]. 2010.
- [39] Orta De Velasquez MTY-N, Yáñez-noguez I, Jimenez-Cisneros B, Luna Pabello VM. Adding silver and copper to hydrogen peroxide and peracetic acid in the disinfection of an advanced primary treatment effluent. *Environ Sci Technol.* 2008;29(11):8.
- [40] Pratt M. Differential response of various spore species to sporicidal disinfectants. Provo, Utah: Brigham Young University [Thesis]; 2007.
- [41] Robert TDW, Winters DR, Harper BG. Final Test Report for the Sporicidal Efficacy of PeraDox on *Bacillus anthracis*. Test Project Report: US Army Developmental Test Command, Division LS;2006 March 2006 Contract No.: WDTC-TR-06-034.
- [42] Maki DGD, Duster M. The promise of simple and total disinfection of hospital surfaces by aerosolization of peroxyacetic acid. Interscience Conference on Antimicrobial Agents and Chemotherapy [Abstract]. 2009.
- [43] Wood JC, Atwood, B. Dry fogging of hydrogen peroxide/peracetic acid for *Bacillus* spore inactivation. EPA Decontamination Research Conference; Nov, 2011; Research Triangle Park, NC:2011.
- [44] Wood JP, Worth Calfee M, Clayton M, Griffin-Gatchalian N, Touati A, Egler K. Evaluation of peracetic acid fog for the inactivation of *Bacillus anthracis* spore surrogates in a large decontamination chamber. *J. Haz. Mat.* 2013; 250-251:61-67.
- [45] Gregersen JP, Roth B. Inactivation of stable viruses in cell culture facilities by peracetic acid fogging. *Biologicals.* 2012; 40(4):282-7.

UNCLASSIFIED

- [46] Krishnan JF, Fey G, Stansfield C, Landry L, Nguy H, Klassen S, Robertson C. Evaluation of a Dry Fogging System for Laboratory Decontamination. Winnipeg, Manitoba: Public Health Agency of Canada 2012.
- [47] Falagas ME, Kopterides P. Risk factors for the isolation of multi-drug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*: a systematic review of the literature. *J. Hospital Infection* 2006;64:7-15.
- [48] Sattar SA, Sabbah S, Springthorpe VS, Theriault S, Krishnan J, Cizik C *et al.* Establishing standards for decontamination of civilian buildings after the release of bioagents. Science and Technology Branch Environment Canada Manuscript, Report Series EE-184; 2011.
- [49] Oie S, Obayashi A, Yamasaki H, Furukawa H, Kenri T, Takahashi M, *et al.* Disinfection methods for spores of *Bacillus atrophaeus*, *B. anthracis*, *Clostridium tetani*, *C. botulinum* and *C. difficile*. *Biol Pharm Bull.* 2011;34(8):1325-9.
- [50] Xue Z, Seo Y. Impact of chlorine disinfection on redistribution of cell clusters from biofilms. *Environ Sci Technol.* 2013;47(3):1365-72.
- [51] Peleg AY, Hooper DC. Hospital-Acquired Infections Due to Gram-Negative Bacteria. *New England J. Med.* 2010;362(19):1804-1813
- [52] Moellering RC, Jr. Vancomycin-resistant enterococci. *Clin Infect Dis.* 1998;26(5):1196-9.
- [53] Cetinkaya Y, Falk P, Mayhall CG. Vancomycin-resistant enterococci. *Clin Microbiol Rev.* 2000;13(4):686-707.
- [54] Altekruze SF, Street DA, Fein SB, Levy AS. Consumer knowledge of food-borne microbial hazards and food-handling practices. *J Food Prot.* 1996;59(3):287-94.

Annex A Glossary of Terms

Antibiotic: a chemical substance, such as penicillin or streptomycin, produced by or derived from certain fungi, bacteria, and other organisms, that can destroy or inhibit the growth of other microorganisms; may also be synthetically derived. Antibiotics sufficiently nontoxic to the host are used in the treatment of infectious diseases.

Antiseptic: a product applied to the surface of a living organism or tissue to prevent or stop the growth of microorganisms by inhibiting the organism or by destroying them.

Bioburden: the number and types of viable organisms that contaminate a piece of equipment/device.

Biocide or Germicide: chemical agents that kill microorganisms. These general terms include disinfectants, antiseptics and antibiotics. Actions may include oxidation, hydrolysis, denaturation or substitution. Suffix *-cide* implies a killing action, whereas *-static* implies the inhibition or prevention of growth of an organism.

Biofilm: an aggregate of microorganisms wherein the cells adhere to each other on a surface. Biofilm formation serves as protection against disinfection.

Bioterrorism: the intentional or threatened use of microorganisms or their products (e.g. toxins) to cause death or disease in humans, animals or crops.

Cleaning: the physical removal of foreign material (e.g. dust, soil) and organic material (e.g. blood, secretions, excretions, microorganisms). Cleaning physically removes rather than kills microorganisms. It is accomplished with water, detergents and mechanical action.

Decontamination: the process of removing foreign material such as blood, body fluids, or radioactivity. It does not eliminate microorganisms but is a necessary step preceding disinfection or sterilization.

Detergent: solutions that disperse and remove soil and organic material from surfaces allowing a disinfectant to reach and destroy microbes within or beneath the dirt. These products reduce the surface tension and increase the penetrability of water, allowing more organic matter to be removed from surfaces. Some disinfectants also have detergent properties (e.g. chlorine compounds and QACs).

Disease: a medical condition associated with specific symptoms and signs. An infectious disease is caused by the invasion and reproduction of a pathogen in the host.

Disinfectant: a product applied to inanimate objects to destroy or irreversibly inactivate microorganisms, fungi and viruses, but not necessarily spores. In most instances, a given disinfectant is designed for a specific purpose and is to be used in a certain manner.

High Efficiency Particulate Air (HEPA) Filter: a filter which has an efficiency of 99.97% in the removal of airborne particles 0.3 microns or larger in diameter.

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High-Level Disinfection: the level of disinfection required when processing semicritical medical equipment/devices. High-level disinfection processes destroy vegetative bacteria, mycobacteria, fungi and enveloped (lipid) and non-enveloped (non-lipid) viruses, but not necessarily bacterial spores. Medical equipment/devices must be thoroughly cleaned prior to high-level disinfection.

Host: an animal or person that harbours an infectious agent.

Indicator: a system that reveals a change in one or more of the sterilization process parameters. Indicators do not verify sterility, but they do allow the detection of potential sterilization failures due to factors such as incorrect packaging, incorrect loading of the sterilizer, or equipment malfunction.

Infection: the invasion of a host's cells/tissues by a pathogen, its multiplication and the reaction of host tissues to the microorganisms and/or their products.

Infectious Agent: See Pathogen.

Infectious Dose: the amount of a pathogen (measured in number of microorganisms or their products) required to cause disease in the host.

Log Reduction: "Log reduction" is a mathematical term (as is "log increase") used to show the relative number of live microbes eliminated from a surface by disinfecting or cleaning. For example, a log reduction of 5 means if 100,000 pathogenic microbes were found on a surface prior to disinfection they would be reduced to 1.

Low-Level Disinfection: level of disinfection required when processing non-invasive medical equipment (i.e., non-critical equipment) and some environmental surfaces. Equipment and surfaces must be thoroughly cleaned prior to low-level disinfection.

Microorganism or Microbe: an organism that is microscopic, too small to be seen unaided by the human eye. It can be a single cell or a multicellular organism. Examples include bacteria, viruses, fungi and prions.

Mycobacterium: a species of bacteria that includes pathogens *Mycobacterium tuberculosis*, the causative agent of tuberculosis, and *M. leprae*, the causative agent of leprosy.

Pathogen: a microorganism (bacteria, virus, fungus, prion or parasite) that causes disease in its host. Also referred to as an infectious agent.

Pathogenicity: the ability of a pathogen to produce an infectious disease in an organism. The outcome of disease is dependent upon the relationship between the resistance of the host and the virulence of the pathogen.

Opportunistic Infection: an infection cause by opportunistic pathogens – those that take advantage of certain situations but do not usually cause disease in a healthy host.

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Reservoir: a place where an infectious agent lives and reproduces in that allows it to be transmitted to the next available host.

Sanitizer: products that reduce the number of microbial contamination on inanimate surfaces to levels that are considered safe from a public health standpoint. Many sanitizers are a formulation of a detergent and disinfectant.

Spectrum of activity: defines the range of pathogens that are sensitive to a disinfectant. For example, a disinfectant with a broad spectrum of activity would be effective against many different pathogens.

Sterilization: the process (physical or chemical) that destroys or eliminates all forms of life, especially microorganisms.

Technology Readiness Level (TRL): a measure used to assess the maturity of evolving technologies during its development through to implementation.

Virulence: the tendency of a pathogen to cause damage to a host. Virulence is the degree (or quantitative measurement) of pathogenicity of a microorganism. Virulence factors include toxins, enzymes and other products that enable pathogen invasion, adhesion and survival.

Virus: a microorganism that can only replicate inside of host cells. A virus consists of genetic material (DNA or RNA) and a protein coat. In addition, a virus may be “enveloped”, in which the protein coat is surrounded by an envelope of lipids, or “non-enveloped.” Whether a virus is enveloped or not influences its resistance to disinfection.

Zoonoses: an infectious disease that is transmitted between species, from animals to humans. Zoonoses depend upon contact with animals or animal products. For example, one can contract rabies from being bitten by an infected animal or tularemia through contact with animal carcasses.

Annex B Chain of Infection

A human, a susceptible host, can be infected by a pathogen when the elements of a chain of infection are present (Figure 1). The necessary factors include the presence of an infectious agent (pathogen), a reservoir, a susceptible host, a mode of transmission, a portal of entry, and a portal of exit, to perpetuate the cycle.

To cause disease an infectious agent must be viable. For this a “reservoir” is necessary. A reservoir is a place that the infectious agent lives and reproduces in that allows it to be transmitted to the next available host or susceptible individual. The mode of transmission occurs from the reservoir of the infection (human host or animal) to a susceptible host by several routes:

- i) contact: directly from person-to-person, indirectly through a non-living object or fomite, or by droplet transmission (mucus coughed or sneezed into the air);
- ii) vehicles: medium carries pathogen that is ingested or inhaled (e.g. water, food, air); or
- iii) vectors: animals that carry pathogens from one host to another without being infected themselves (e.g. west Nile virus spread by mosquitoes).

To infect a susceptible host, the infectious agent must pass from the reservoir and gain access to the potential host. Through the route of entry the infectious agent then enters the particular cell type of the host in which it will live (it must adhere to and penetrate those cells and evade host defense mechanisms). Once established in hospitable cells, the infectious agent then propagates. This propagation of the microorganism causes disease within the host. To perpetuate its survival the microorganism must also exit the host, be transmitted to another potential host and start the cycle again.

Humans are vulnerable to entry of infectious agents through several portals. Portals of entry include: the respiratory tract (inhalation of airborne microorganisms); gastrointestinal tract (ingestion of the microorganism with food and water), and through close contact (passage of organisms by salivary, skin and genital contact - impenetrable by most organisms unless the surface is broken). Inhalation and ingestion are the most common routes of entry of infectious agents. At the other end of the cycle, these agents typically exit through the same portals, either the respiratory systems through a cough or sneeze or via the gastrointestinal tract in feces.

Human factors impact the virulence of a microorganism, especially when considering opportunistic microorganisms. One’s susceptibility to an infectious agent, or the ability to fight off a pathogen, is affected by several factors including nutrition, stress, genetic background, presence of co-morbid disease, environment, and whether the microbe has been encountered before (immune memory).

Annex C Infectious Agents

Common Agents of Hospital Acquired Infections:

- 1) *Pseudomonas aeruginosa* is a pathogen commonly encountered in the hospital environment and is a common cause of HAI. This species is tolerant to a wide variety of living conditions and thrive in wet, damp places such as sinks, drains or mop buckets. The characteristic that seems to confer protection against environmental factors is the ability to form a biofilm (a group of connected cells) and attach to a surface. *P. aeruginosa* is a serious problem in hospitals because of its ability to survive in biofilms and its resistance to cleaning and disinfection [51]. *P. aeruginosa* exploit breakdowns in host defense to cause opportunistic infections and these bacteria will infect just about any compromised tissue. *P. aeruginosa* is the most common cause of infections of burn injuries and can cause wound infections, bacteremia, pulmonary disease (especially in those with cystic fibrosis), urinary tract infections, and soft tissue infections. Among its arsenal of assault tactics, *P. aeruginosa* produces toxins and a variety of enzymes that allow it to invade and spread through cells and into tissues (proteases, hemolysin, lecithinase, elastase, coagulase, and DNAase). The presence of flagella, whip-like extensions, allows *P. aeruginosa* motility in liquids.
- 2) *Staphylococcus aureus* can be found as part of one's normal skin flora. However, *S. aureus* can cause a range of opportunistic infections from minor infections (boils) to life-threatening disease (pneumonia, meningitis, toxic shock syndrome and sepsis). *S. aureus* produce enzymes that aid in their tissue spread and invasion and they produce toxins that cause harmful effects. Methicillin-resistant *Staphylococcus aureus*, or MRSA, is a strain of *S. aureus* that has become resistant to most antibiotics used to treat staphylococcal infections. MRSA infections are often acquired in the hospital or in other health care facilities because of the predominance of weakened immune responses in these institutions. Transmission of MRSA, like *S. aureus*, is through either human-to-human contact or through contact with a surface that has been recently touched by a person infected with MRSA.
- 3) *Enterococcus* species are tolerant of a wide range of living environments including extreme temperature (10-45°C), pH (4.5-10) and salt concentrations. Enterococci are normal inhabitants of the human gastrointestinal tract with more than a dozen recognized species of enterococcus, only two of which cause infection in humans. The most common infections caused by enterococci are urinary tract infections, followed by surgical wound infections and endocarditis. Enterococcus species are not very pathogenic, they do not produce enzymes or toxins like *P. aeruginosa* or *S. aureus*, but all enterococci are resistant to β -lactam agents (penicillin family antibiotics), which is what makes these microbes formidable pathogens [51]. Since the 1980s particularly virulent strains of enterococci have developed resistance to vancomycin, vancomycin-resistant enterococci (VRE), and have emerged as a significant cause of HAI [52][53]. Spread of VRE occurs through contact, contact with either infected patients, uninfected carriers or with contaminated environmental surfaces.
- 4) Food-borne illness (FBI), sometimes called "food poisoning," results from the consumption of food or drink contaminated with an infectious agent or its product (e.g. toxin). Symptoms of food-borne illness include nausea, vomiting, diarrhea, stomach cramps, fever, headache and dizziness. There are greater than 200 diseases transmitted through food contamination. Food-borne microorganisms present in foods of animal origin can cause disease when the product is improperly prepared or eaten raw, such as raw meat, fish and eggs. Influence of food safety awareness, food handling experience and age all play a role [54]. However, these instances of food related illness are limited in scope, typically within a household.

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- 5) The *Salmonella* species of bacteria are gram-negative, anaerobic, motile rods that are among the normal gastrointestinal bacterial population but these microbes may cause gastrointestinal illnesses in humans. *Salmonella* species are a common cause of food-borne illness. Infection is caused by ingestion of the organisms in food or water contaminated with human or animal feces, or through ingestion of insufficiently cooked poultry, milk, eggs and dairy products. Several food recalls over the last few years have demonstrated the ability of *Salmonella* to grow on the surface of fruits and vegetables, including alfalfa sprouts, spinach, tomatoes and lettuce. Reported cases of food poisoning after ingestion of contaminated produce emphasize the ability of salmonella to cause infection via this route. Salmonella infection may occur in several forms: acute gastroenteritis or food poisoning characterized by vomiting and diarrhea; typhoid fever; or bacteremia. Individuals may also exist in a carrier state that follows *Salmonella* infection during which there are no symptoms seen of the infection, but contamination is possible.
- 6) *Escherichia coli* are rod-shaped bacteria that are often part of the normal gastrointestinal flora. *E. coli* strains have been associated with a wide range of diseases and infections including neonatal meningitis, and gastrointestinal and urinary tract infections in all age groups. Each serotype is a classification based on carbohydrates attached to the bacterial cell wall (O, H and K antigens). There have been more than 700 serotypes of *E. coli* identified. Among these, the most relevant pathogen in humans is *E. coli* strain O157:H7. *E. coli* O157:H7, an enterohemorrhagic strain, is associated with hemorrhagic diarrhea and hemolytic uremic syndrome (HUS). This strain is highly virulent and requires only a small infectious dose to cause disease due to the production of Shiga toxin. The classic illness is characterized by a watery diarrhea that progresses to a bloody diarrhea and crampy abdominal pain, with or without low-grade fever. Further, *E. coli* O157:H7 can also be a cause of severe food poisoning.
- 7) Infection with *E. coli* O157:H7 follows ingestion of contaminated food or water, or oral contact with contaminated surfaces. Consumption of undercooked ground beef, unpasteurized milk and juice, raw produce and contact with infected live animals are causes of infection. Healthy cows can harbour *E. coli* O157:H7 in their gastrointestinal tract. Flesh can become contaminated during slaughter and butchering, and organisms can be thoroughly mixed into beef when it is ground into hamburger. Bacteria present on the cow's udders or on equipment may get into raw milk. Fecal-oral transmission is another major route through which *E. coli* strains cause disease having passed through the gastrointestinal tract of the cows and spread through the feces. Recent outbreaks of *E. coli*-associated FBI were caused by contaminated spring mix and spinach. Of note, *E. coli* water contamination was the cause of the Walkerton tragedy in 2000 in which nearly 2,300 people fell sick because of improper water treatment practices.

Common Agents of Food-borne Illness:

- 1) **Norwalk virus** is a non-enveloped, single-stranded RNA virus that is part of the Calicivirus family and the Norovirus genus. The virus is very contagious and spread through direct contact with infected people, contaminated water or food or indirectly through environmental surfaces. It is the cause of acute gastroenteritis and is thought to account for 50% of FBI. The onset of symptoms typically occurs within 12-48 h of exposure and last for 24-60 h and include pain, nausea, diarrhea and fever. The foods commonly involved in outbreaks include leafy greens (e.g. lettuce), fresh fruits (e.g. strawberries), and shellfish (e.g. oysters).
- 2) ***Clostridium perfringens*** is a gram-positive, spore-forming bacteria that is responsible for an estimated 1,000,000 cases of FBI each year. The onset of symptoms is usually within 12-72 h of *C. perfringens* ingestion and includes watery diarrhea and abdominal cramps, but not fever or vomiting, lasting 4-7 days. The spores of *C. perfringens* survive high temperatures and then germinate to grow

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bacteria that cause infection after ingestion. *C. perfringens* infections are usually associated with the preparation of large quantities of food, meat or poultry, that is kept warm (e.g. in a hospital cafeteria or at catered events).

- 3) *Staphylococcus aureus* is a gram-positive bacterium that produces several types of toxins. Ingestion of the toxins is the cause of Staphylococcal “food poisoning,” a gastrointestinal illness presenting with nausea, vomiting, abdominal cramps and diarrhea lasting 24-48 h. *S. aureus* FBI is transmitted directly by ingestion of toxins in food or indirectly by hands that have been contaminated with *S.aureus* toxins. These toxins are resistant to heat and are not destroyed by cooking and it can take as little as 1 microgram to cause Staphylococcal food poisoning.
- 4) *Listeria monocytogenes* is a gram-positive bacterium responsible for Listeriosis, the most virulent FBI and the leading cause of FBI-related death. Listeriosis primarily affects the elderly, pregnant women, newborns and immunocompromised people. In pregnant women symptoms include fever, fatigue and aches but can cause miscarriage, stillbirth, premature birth or life-threatening infection in the newborn. Common symptoms in non-pregnant women are fever and muscle aches but may also include headache, stiff neck, confusion, loss of balance and in some cases convulsions. Recent outbreaks of Listeriosis were associated with ingestion of contaminated cheese and cantaloupes. Its prevalence is in part due to its ability to grow at refrigeration temperatures.

Infections Agents of Outbreaks in Livestock and Poultry:

- 1) **Foot and Mouth Disease (FMD)** is a non-enveloped single-stranded virus of the *Picornavirus* family. It is highly contagious, and sometimes fatal, disease of cloven-hoofed animals (e.g. domestic cattle, sheep, goats and pigs). FMD manifests within 2-8 days but hosts are infectious prior to the onset of symptoms and up to 7-10 days afterwards. Symptoms include excessive salivation; sores and blisters on the feet nose, lips and in the mouth; and shivering. FMD spreads by direct contact with infected animals, their fluids and scabs from their blisters have high concentrations of infectious virus. It can also spread via blood, saliva, milk and manure or indirectly by contaminated equipment, clothing, or footwear.
- 2) **Bovine Spongiform Encephalopathy (BSE)** and Scrapie are prion diseases and although not contagious, there is no treatment and they have a far-reaching decontamination process. BSE and Scrapie are transmissible spongiform encephalopathies (TSE), as is Creutzfeldt-Jakob Disease (CJD) in humans; these are a group of progressive brain and nervous system diseases caused by prions. Prions are abnormal or misfolded proteins in the brain that cause encephalopathy – a disease that affects the function of the brain and nervous system. They are slow developing and can take 3-6 for BSE, and 1-8 years for Scrapie, after exposure for signs of onset to develop. Spread of TSE’s is through feeding of infected meat and bone meal . Symptoms of TSE’s are variable and progressive relating to nervous system function. Prions are resistant to normal inactivation procedures including most disinfectants and heat.
- 3) *Mycoplasma mycoidis* are bacteria of the Mycoplasma family – a family that lacks a cell wall, unlike gram-positive or gram negative bacteria that have cell walls with specific staining characteristics. *M. mycoidis* is a contagious lung disease of small ruminants responsible for contagious bovine pleuropneumonia.

Biological Agents of terrorism:

- 1) **Botulinum toxin (BTX)** and **Ricin toxin** are both chemicals of biological origin, i.e. non-infectious agents not transmissible from person to person that could be used for bioterrorism.
 - BTX can be produced by *Clostridium botulinum*, *C. baratii* and *C. butyricum*, and is the causative agent of botulism. The spores of these bacteria can be found naturally in soil like *B. anthracis*. BTX is a neurotoxin that causes paralysis of the respiratory muscles and leads to respiratory failure and eventually death. BTX is the most potent naturally produced toxin known with a lethal dose as little as 0.7-0.9 µg.
 - Ricin is a toxin that is produced from castor beans and can be made from the waste material or mash left over from the production of castor oil (~1,000,000 tons of castor oil is produced worldwide each year). Ricin toxin is very potent and stable. The effects of ricin depend on the route of exposure: with sufficient doses inhalation can result in acute pulmonary disease and ingestion in gastrointestinal hemorrhage, both of which can be fatal.
 - BTX is classified as a category A agent by the CDC, whereas ricin is a category B agent. This difference is due to the difference in potency of the toxins and the requisite mass of each agent to cause morbidity and mortality – a lethal dose causing 50% mortality of ricin is an estimated 8 tons to affect a 100km² area, 5-fold more than that of BTX required for same effect.
- 2) ***Yersinia pestis*** is a gram-negative bacterium that is the causative agent of plague, a zoonotic disease of rodents. Historically, when rodent populations die off, the fleas are forced to feed off humans which can cause plague epidemics. There are three forms of plague that manifest, the form is largely dependent upon the route of transmission. Bubonic plague is the most common presenting with swollen, tender lymph nodes and generally results from transmission by fleas, although septicemic plague may result from flea spread as well. Septicemic plague is generally contracted from handling contaminated tissue or fluids of infected animals. Pneumonic plague is the result of inhalation exposure and is the predominant form expected from aerosolization of *Y. pestis* for biological warfare. It is highly lethal with a low infectious dose and highly contagious with the potential for secondary spread through person to person transmission (e.g. cough droplets). The World Health Organization (WHO) estimates that 50kg of aerosolized plague released over a city of 5 million people could result in 50,000 cases of plague with approximately 36,000 deaths.
- 3) **Tularemia** is caused by *Francisella tularensis*, a gram-negative bacterium that can survive for weeks at low temperature in water or moist soil and in decaying carcasses. Small mammals are the natural reservoir (e.g. mice, squirrels, rabbits). Primary transmission is through vectors (e.g. ticks and deer flies), but can be spread through handling infected animal carcasses, consumption of contaminated food or water and from person to person contact via inhalation of infected particles. There are many forms of tularemia that depend on the route of transmission including pneumonic, typhoidal, septic, ulceroglandular, glandular, oculoglandular and oropharyngeal. Symptoms of all forms include fever, headache, body aches and malaise which are detectable 3-5 days after exposure. *F. tularensis* is very infectious (as little as 10 organisms can cause disease), often fatal and it can be aerosolized; all properties that mitigate its use as a biological agent for warfare.
- 4) **Viral hemorrhagic fevers (VHF)** are caused by a diverse group of viral organisms capable of causing zoonotic disease associated with fever and bleeding disorders. There are 4 families of viruses responsible for causing VHF:
 - *Filoviridae* (e.g. Ebola, Marburg viruses)

- *Arenaviridae* (e.g. Lassa fever virus)
- *Bunyaviridae* (e.g. Crimean Congo hemorrhagic fever virus, Hanta virus)
- *Flaviviridae* (e.g. Dengue fever virus)

As a group, they are highly infectious viruses capable of high rates of morbidity and mortality. Several can be transmitted from person to person including Ebola, Marburg, Lassa and Crimean Congo VHF. All present with symptoms of fever, rash, body aches, headache and fatigue with internal and external bleeding later in the course of the infection. Several VHFs were reportedly developed as aerosol weapons, a weapon with the potential to affect both human and animal populations.

- 5) *Bacillus anthracis* is the causative agent of anthrax, a disease whose natural reservoirs include grazing animals such as cattle. *B. anthracis* is a very large, gram-positive rod-shaped bacterium that forms spores that can be found living naturally in soil. The pathogenicity of *B. anthracis* is mainly due to its ability to evade the host's immune system via the formation of a capsule. Damage to the host occurs because of the production of anthrax toxins.

B. anthracis infection can occur through contact with, or inhalation or ingestion of the bacteria or its spores. The type of anthrax is a result of the route of entry: cutaneous anthrax infects the skin (contact), inhalation anthrax infects the lungs (inhalation), and gastrointestinal anthrax infects the gastrointestinal tract (ingestion). Naturally acquired anthrax in humans is extremely rare, but may occur from inhalation of contaminated dust or by contact with animals carrying the spores. The most common form of anthrax is cutaneous anthrax wherein *B. anthracis* spores come into contact with injured skin or mucous membranes, resulting in infection of the skin. The characteristic symptom is the formation of painless black eschars at the site of infection. Anthrax contracted by inhalation of *B. anthracis* spores is associated with the most severe health consequences. Inhalation anthrax progresses rapidly to a systemic hemorrhagic disease that is often fatal. The possibility of creating aerosols containing anthrax spores has made *B. anthracis* a chosen weapon of bioterrorism. Spores of *B. anthracis* can be produced and stored in a dry form and remain viable for decades in storage or after release. As an agent of bioterrorism it is expected that a cloud of *B. anthracis* spores would be released at a strategic location to be inhaled by a large number of individuals. The potential area affected and the number of people that could be affected in an urban center is huge and the effects would be devastating if appropriate countermeasures, which would have to be available in large amounts, were not implemented in time.

The high mortality rate from anthrax infections can be attributed chiefly to the difficulty in diagnosing anthrax. If antibiotic treatment is not given before the onset of symptoms, the prognosis for recovery is very poor. Toxins secreted by replicating, fully germinated bacteria cause symptoms. To prevent symptoms, antibiotics must be administered soon after exposure, before the replicating bacteria have had a chance to release toxins. Initial symptoms of exposure, regardless of route of entry, may include general symptoms such as fever, nausea, malaise, headache, and sweating. These symptoms can last from a few hours, to a few days [10]. Because of the need for early diagnosis, its general flu-like symptoms and the resistance of spores to disinfection methods, the spores of *B. anthracis* make an ideal agent for biological terrorism.

Annex D Disinfection Approaches

D.1 Physical Disinfection

D.1.1 Heat

Moist heat, in the form of saturated steam under pressure, is the most reliable sterilization method. Moist heat destroys microorganisms by coagulating and denaturing enzymes and structural proteins. Steam sterilization is non-toxic, inexpensive, easy to use, rapid, sporicidal, and rapidly penetrates fabrics. However, steam sterilization is destructive and is limited to items that can withstand these conditions. An autoclave, which utilizes moist heat under steam pressure (steam under 1 atm or 15 psi), can achieve temperatures of 121-132 °C. Generally it takes 30 minutes to achieve complete sterilization under these conditions. Autoclaves are routinely used to sterilize laboratory equipment, such as glass beakers.

D.1.2 Filtration

Filtration methods, although not based on killing the microorganism, can be used to remove bacteria, yeasts and molds from liquid or air. In order to remove bacteria, the pore size of the filter or membrane must be uniform throughout and small enough to trap bacteria and aerosols (by impaction, diffusion, interception and sedimentation). The most common application of filtration, pore size < 0.2 µm, is in the sterilization of heat-sensitive solutions (such as vaccines or antibiotics). Filtration of air is accomplished with the use of High-Efficiency Particulate Air (HEPA) filters which have a pore size of ~0.3 µm. HEPA filters are used in laboratory hoods and in quarantine patient rooms.

D.1.3 Radiation

Radiation may be either an ionizing or non-ionizing form. Ionizing radiation, or gamma rays (e.g. Cobalt 60) or electron beams, are of high energy and short wavelengths. Ionizing radiation is used commonly to sterilize disposable medical supplies, tissue for transplantation and pharmaceuticals. This method is expensive and items for sterilization must often be transported to the site of radiation, but it is suitable for large quantities of devices. Some deleterious effects have been noted on electronics and patient sensitive medical devices, e.g. knee bearings for transplantation have been associated with delamination.

Non-ionizing radiation, or ultraviolet (UV) radiation, is of low energy and longer wavelengths (328-210nm). Inactivation of microorganisms occurs from the DNA/RNA damage induced by UV rays. Bactericidal effects occur at 240-280nm and can be achieved with mercury vapor lamps (emission at 254 nm). UV radiation is bactericidal, viricidal and can kill spores with appropriate exposure times. However, the effectiveness of UV radiation for sterilization is influenced by the presence of organic matter; wavelength of the light source; type of suspension; temperature; type of microorganisms; and UV intensity which is also affected by distance and dirt on the tubes. Ultraviolet radiation is used in healthcare in the laboratory (biological hoods to disinfect surfaces).

D.2 Chemical Disinfectants

D.2.1 Alcohols

Alcohols are broad-spectrum antimicrobial agents that act by denaturing cell membrane proteins and causing cell lysis. Alcohol solutions in water, 60-90% (volume/volume), of ethanol or isopropanol are most commonly used for hospital disinfection. Alcohols are considered fast-acting tuberculocidal, bactericidal and fungicidal agents but they are not sporicidal. Ethanol is viricidal, whereas propanol is only partially viricidal as it is not effective against non-enveloped viruses. Alcohols are used for surface disinfection, topical antiseptic and hand sanitizing lotions because they are fast acting, do not leave residue and are fairly inexpensive. Alcohols are also compatible with other disinfectants (quaternary ammonium compounds, phenolics and iodine) and can be used in combination depending on need. Disadvantages include their flammable nature, inactivity in the presence of organic matter, toxicity if ingested, eye irritant, and quick evaporation time which limits contact time and may inhibit killing as a result.

D.2.2 Aldehydes

Aldehydes are highly effective, broad-spectrum disinfectants that typically achieve sterilization by denaturing proteins and disrupting nucleic acids. The most commonly used agents are formaldehyde and glutaraldehyde. Aldehydes are effective against bacteria, fungi, viruses, mycobacteria and spores but are limited by their adverse effects. These agents are non-corrosive to metals, rubber, plastic and cement, but are highly irritating and toxic to humans with contact or inhalation. Exposure hazards and potential carcinogenic effects have limited the use of formaldehyde and glutaraldehyde. Personal protective equipment must be worn when using these chemicals.

Formaldehyde is used as a surface disinfectant and a fumigant and has been used to disinfect air-tight buildings, but the building must remain closed for at least 24 hours after treatment. Formaldehyde penetrates bacterial spores and has been traditionally used to sterilize medical equipment in combination with alcohols. The efficacy of formaldehyde is dependent on relative humidity and temperature (optimum 70% and 14°C, respectively).

Glutaraldehyde (e.g. CIDEX) is a high-level disinfectant primarily used to disinfect medical equipment (e.g. endoscopes), but can provide sterilization at prolonged contact times. A 2% concentration is used for high-level disinfection. Its efficacy and sporicidal activity is highly dependent on a neutral to alkaline pH (>7) and high temperatures (>37° C). Glutaraldehyde is considered more efficacious in the presence of organic matter, soaps and hard water than formaldehyde.

D.2.3 Chlorine Compounds

Chlorine compounds are broad-spectrum compounds that are considered low toxicity, low cost and easy to use. Chlorine compounds function through their electronegative nature to destroy the cellular activity of proteins. This results in metabolic inhibition of the organism. Chlorine compounds are effective against bacteria, enveloped and non-enveloped viruses, mycobacteria, and fungi. At elevated concentrations and with appropriate contact times (30 min.), chlorine compounds can be sporicidal. They lose potency over time and are not active at high temperatures (>43°C) or high pH (>9). These compounds lose activity in the presence of organic debris, sunlight and some metals, and should be applied to thoroughly cleaned surfaces for disinfection.

Sodium hypochlorite, or household bleach, is one of the most widely used disinfectants for hard surfaces and blood spillages. Bleach is effective against HIV or hepatitis B virus in blood. Biocidal activity is determined by the amount of available chlorine in solution. (Commercial bleach contains 5.25% sodium hypochlorite in aqueous solution, 50,000 ppm available chlorine). Rapid sporicidal action can be obtained around 2500 ppm, however this concentration is very corrosive. High concentrations are also irritating to the mucous membranes, eyes and skin. Hypochlorites should never be mixed with acids or ammonia because it results in the release of toxic chlorine gas.

D.2.4 Iodophors

Iodine complexes have increased solubility and sustained release of iodine compared with iodine, a useful antiseptic. Iodophores are a combination of iodine (0.5-5%) and a neutral polymer carrier. Iodophors are also less irritating, non-staining and more stable than iodine in its pure form. Iodophors are effective against bacteria and both lipid and non-lipid viruses, but do not have killing activity against *Mycobacterium* or spores. Iodine compounds function by denaturing proteins to interfere with the enzymatic systems, which results in metabolic inhibition of the organism. Proiodine is a commonly used antiseptic for cleaning the surface of the skin.

D.2.5 Oxidizing Agents

Oxidizing agents are broad-spectrum, peroxide based compounds that function by producing destructive hydroxyl-free radicals that act on membrane lipids, DNA and by denaturing the proteins of microorganisms. Peroxygen compounds vary in the microbicidal activity, but are considered effective on hard surfaces and equipment. In their diluted form, these agents are relatively safe but may be irritating to skin and eyes.

Hydrogen peroxide (H_2O_2 ; 5-20%) is bactericidal, viricidal (non-enveloped viruses may be resistant), fungicidal and at high concentrations sporicidal. Its activity against mycobacteria is limited. A 7.5% hydrogen peroxide solution (e.g. Steris Resert XL HLD) is used in some healthcare settings as a sterilant and high-level disinfectant. It is a stable solution and is environmentally friendly.

Peracetic acid (PAA) is a strong oxidizing agent. PAA solutions are considered to be a more potent disinfectant than hydrogen peroxide. PAA is sporicidal, bactericidal, viricidal and fungicidal at low concentrations and is also environment friendly. PAA can be used as a disinfectant for medical devices and as an environmental surface sterilant.

Combination solutions of H_2O_2 and PAA (e.g. Minncare Cold Sterilant or Steriplex Ultra) are found to be more effective, particularly against glutaraldehyde-resistant mycobacteria.

D.2.6 Phenols

Phenols are broad-spectrum disinfectants that function by denaturing proteins and inactivating membrane-bound enzymes to alter the cell wall permeability of microorganisms. At low concentrations phenols cause cell lysis by interaction with cell wall enzymes. At high concentrations phenols cause coagulation. Phenols can be coal-tar derivatives or synthetic formulations and usually have a milky or cloudy appearance when added to water and a strong pine odour. Pine-Sol is an example of a phenol. Phenols are typically formulated in soap solutions to increase their penetrative powers. At 5% concentrations phenols are bactericidal, tuberculocidal, fungicidal and viricidal for enveloped viruses. Phenols are not effective against non-enveloped viruses or spores. Phenols do maintain activity in hard water and in the presence of

organic matter and have some residual activity after drying. Phenolic disinfectants are generally safe for humans but prolonged exposure to skin may cause irritation. Concentrations over 2% are highly toxic to animals. Two phenol derivatives used commonly in hospital disinfectants are orthophenylphenol (e.g. Amphyl, Reckitt Benckiser) and ortho-benzyl-parachlorophenol (e.g. Clorox Disinfectant Cleaner, The Clorox Company).

D.2.7 Quaternary Ammonium Compounds

Also known as “quats” or QACs, these compounds are cationic detergents that are attracted to the negatively charged surfaces of microorganisms, where they irreversibly bind cell membrane phospholipids and denature proteins impairing permeability. The result is lysis of the cell. QACs can be from different “generations” with differing chemistry, foaming potential and tolerance to organic loads. QACs are bactericidal, fungicidal and viricidal (enveloped viruses), but are not effective disinfectants against non-enveloped viruses, mycobacteria or spores. QACs have some residual effect on surfaces, for a brief time, and are used to disinfect non-critical medical instruments (e.g. blood pressure cuffs), hard-surface cleaning and deodorization. They are more active at neutral to slightly alkaline pH but lose their activity at pH <3.5. QACs are considered stable in storage but are easily inactivated by organic matter, detergents, soaps and hard water.

D.2.8 Metal Ions

Heavy metal ions are natural antimicrobials effective against molds, fungi, algae and microbes. Heavy metals (e.g. silver and copper) affect growth, morphology and metabolism of microorganisms. The efficacy of these ions is dependent upon pH, ionic strength, valence, temperature and the presence of oxygen and light. Examples of routine use of silver and copper for antimicrobial activity include surfaces or instruments made of copper or stainless steel, nanoparticle treatments, and in water treatment.

Silver: The antimicrobial mechanism of action for silver is due to the binding of disulphide (S-S₂) and sulfhydryl (-SH) groups of proteins of the cell wall, interrupting cell metabolic processes and leading to cell death. Silver inhibits bacterial growth, cell division and damages the cell wall. Silver has the highest level of antimicrobial activity of heavy metals and is effective against bacteria, viruses, molds and fungi.

Copper: Copper is toxic to antimicrobial cells due to its capacity to catalyze oxidative damage. Copper has a broad spectrum of activity similar to that of silver.

List of Symbols/Abbreviations/Acronyms/Initialisms

AI	Avian Influenza
BSE	Bovine Spongiform Encephalopathy
CASCAD	Canadian Aqueous System for Chemical/Biological Agent Decontamination
CBRN	Chemical, biological, radiological or nuclear event
C & D	Cleaning and disinfecting
CDC	US Centers for Disease Control
CF	Canadian Forces
CFIA	Canadian Food Inspection Agency
CFU	Colony Forming Unit
CI	Complete Inactivation
CJD	Creutzfeldt-Jakob Disease
CSS	Centre for Security Science
CSSP	Canadian Safety and Security Program
DBP	Disinfectant by-product
DFS	Dry Fogging System
DMBAC	Dimethylbenzylammonium chloride
DNA	Deoxyribonucleic Acid
DRDC	Defence Research and Development Canada
EMS	Emergency Medical Services
FMD	Foot and Mouth Disease
FS	Formaldehyde Steam
GCD	Gaseous Chlorine Dioxide
HAI	Hospital Acquired Infection
HVAC	Heating Ventilation and Air Conditioning
LR	Log ₁₀ Reduction
MVM	A mouse parvovirus
MRSA	Methicillin Resistant Staphylococcus aureus
OPS	Ottawa Paramedic Service
PAA	Peroxyacetic acid or peroxygen acetic acid
PHAC	Public Health Agency of Canada
PPE	Personal Protective Equipment
QAC	Quaternary Ammonium Compounds

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RCMP	Royal Canadian Mounted Police
RMCC	Royal Military College of Canada
RNA	Ribonucleic Acid
SARS	Sudden Acute Respiratory Syndrome
SOP	Standard Operating Procedure
TRL	Technology Readiness Level
US	United States
USD	US Dollar
UV	Ultraviolet
VHF	Viral Hemorrhagic Fever
VHP	Vaporized Hydrogen Peroxide
VRE	Vancomycin Resistant Enterococcus
WHO	World Health Organization